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ENDOCRINOLOGY

VOLUME 59 JULY, 1956 NUMBER 1

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TURNOVER OF THYROID HORMONE IN COLD-EXPOSED RATS DETERMINED BY RADIOACTIVE IODINE STUDIES[1]

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Department of Physiology and Biophysics, University of Washington School of Medicine, Seattle, Washington

SMALL mammals subjected to low environmental temperatures for short periods (3–21 days) exhibit alterations in the thyroid gland. Although such changes as increased gland weight (1, 2), cellular hypertrophy (3, 4, 5), and increased radioiodine (I^{131}) content 2 hours after isotope injection (5, 6, 7) have been interpreted as indicating increased functional activity of the gland, they are relatively indirect measures of hormonal secretion rates. However, more direct parameters of hormonal output, such as biologic decay (8) and hormone utilization or requirement (9) are consonant with these interpretations, and suggest that secretion of thyroid hormone increases following exposure to cold. The increased metabolic rate of small mammals placed at low temperatures (1, 10, 11) appears related to these changes in the functional capacity of the thyroid gland.

After animals have been exposed to 5° C for 60 days or more their metabolic rate remains markedly higher than that of unexposed animals, whereas studies of the thyroid gland including cell height (3) and I^{131} content (6, 7) suggest that thyroid function decreases to pre-exposure levels. This discrepancy may result from production of a more active hormone, a change in sensitivity to the hormone, or a maintenance of secretion not detected by these methods.

To test the third hypothesis, that secretory rate is not revealed by methods previously applied, thyroid hormone secretion was estimated by determining the release of radioiodine from the thyroid and the conversion ratios in rats exposed to cold for periods of 1 to 180 days. The results were compared with those obtained by measuring the thyroid weights and I^{131} uptakes in the same group of animals.

Received December 14, 1955.

[1] Aided by Contract AF 33(038)-422 and Contract AF 18(600)-1467 between the University of Washington and the Alaskan Air Command, Arctic Aeromedical Laboratory, Ladd Air Force Base, Alaska.

METHODS

Male Wistar and Sprague-Dawley rats weighing 150–400 gm. were housed in individual cages, fed Purina rat chow, and given water without restriction. The experimental animals were maintained for various periods in a cold room regulated at 5° ±1° C and control animals were kept at 26° ±2° C. In all experiments the cold-adapted rats and the controls were the same age, however differences in weight between controls and experimentals developed because the animals exposed to cold gained weight at a slower rate.

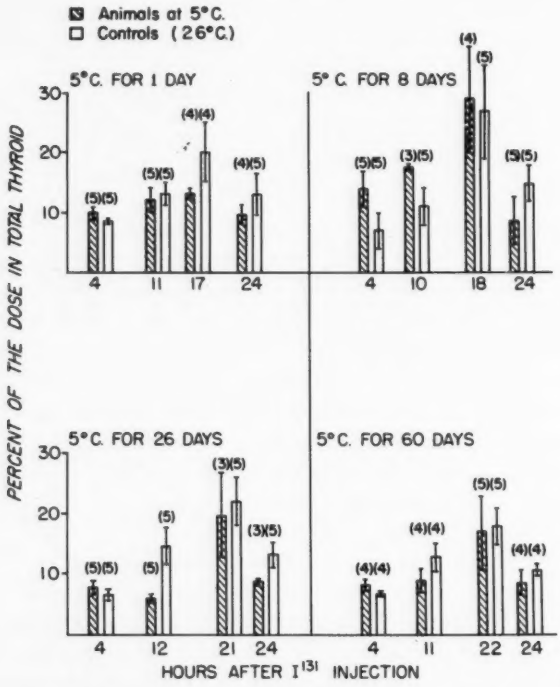
Animals at 5°C.
Controls (26°C.)
5°C. FOR 1 DAY
5° C. FOR 8 DAYS
5°C. FOR 26 DAYS
5°C. FOR 60 DAYS
PERCENT OF THE DOSE IN TOTAL THYROID
HOURS AFTER I131 INJECTION

FIG. 1. Percentage of dose of I^{131} in total thyroid glands of control rats and rats exposed to 5° C for 1, 8, 26 and 60 days. Animals sacrificed at four intervals 4–24 hours after intraperitoneal injection of I^{131}. One standard deviation is shown on either side of mean. Number of animals per group indicated in parenthesis at top of bar.

Lights in the cold room, automatically regulated, were set to approximate the normal day-night condition of the room in which the controls were housed. Carrier-free radioactive iodine was obtained from Abbott Laboratories.

PBI Studies. Animals from groups exposed 1, 8, 26, and 60 days, along with the corresponding control animals, were sacrificed at intervals of 4, 12, 18–22, and 24 hours after intraperitoneal injection of 25 microcuries of radioiodine. The number of rats killed at each time interval is indicated in Figures 1 and 2. During the interval between injection and sacrifice, the rats were kept in individual cages and urine was collected into 20% sodium hydroxide. Animals were allowed water, but food was withheld except from animals sacrificed 24 hours after injection.

At the time of sacrifice, the animals were anesthetized with sodium pentobarbital and blood was obtained by cardiac puncture. The thyroid glands were removed and weighed on a Smith-Roller Torsion balance. Urine in the bladder was added to that collected during the interval between injection and sacrifice.

For estimation of serum protein-bound iodine^{131} (PBI^{131}) the blood was allowed to clot at 0° C and a 2 cc. aliquot of serum was precipitated with trichloracetic acid according to the method of Botkin and Jensen (12). Radioactivity was sampled by using a well-type Texas (Welch-Allyn) geiger counter and an electronic scaler (Nuclear model 163). Duplicate or triplicate counts of 1 minute each were made on each sample and

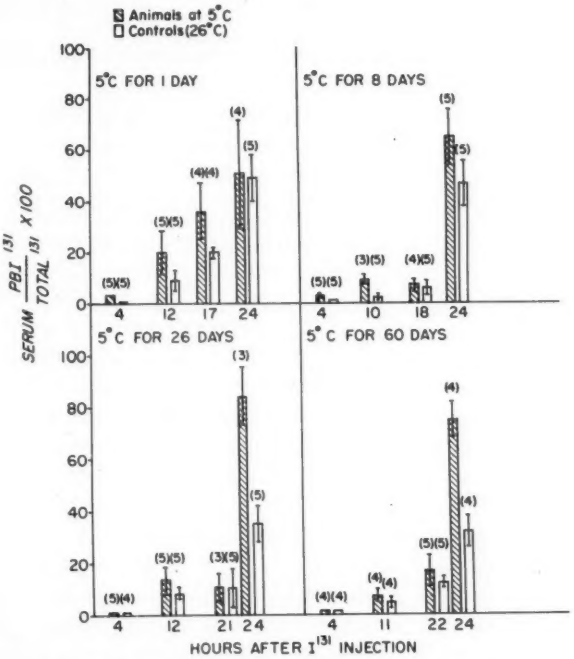

FIG. 2. Conversion ratios ($PBI^{131} \times 100\% / Total^{131}$) for control and experimental animals exposed to 5° C for 1, 8, 26, and 60 days, determined at 4 intervals 4–24 hours after intraperitoneal injection of I^{131}. One standard deviation shown on either side of mean. Number of animals per group indicated in parenthesis at top of bar. Absolute values for PBI^{131} and total I^{131} are shown in Table 3.

averaged. The radioactivity of each sample was compared with that of a standard solution which had been prepared at the time of injection and contained a known percentage of the injected dose. Total serum I^{131} was estimated by counting 1 cc. of serum; the percentage of total serum I^{131} in the protein-bound form was then calculated. The I^{131} in the thyroid gland was determined by counting suitable aliquots of hydrolyzed gland, and the radioactivity of urine by counting an aliquot of urine.

Biologic Decay Studies. Animals for these studies were maintained at 5° C for periods up to 6 months. After an intraperitoneal injection of 1–2 microcuries of I^{131}, the radioactivity over the thyroid region was determined by means of a probe-type G-M tube

TABLE 1. MEAN TOTAL BODY AND THYROID WEIGHTS OF EXPOSED AND CONTROL ANIMALS (GM.)

Exp. temp.		Time in cold			
		1 day	8 days	26 days	60 days
5° C	Body Wt.	176 ± 18*	187 ± 19†	199 ± 18†	252 ± 40†
	Thyroid Wt.	14	12	12	14
26° C	Body Wt.	184 ± 19	224 ± 24	276 ± 16	332 ± 35
	Thyroid Wt.	13	12	14	19

* One standard deviation.
† Significant at 5% when compared to corresponding control animals at 26° C.

(Radiation Counter Tube Mark 1 model 13). Light ether anesthesia was used to immobilize the animals during counting. Counts made 12 or 24 hours after the injection were taken as the zero time from which the biologic half-life was calculated. Thereafter, counts were made daily, or every other day, until the radioactivity had fallen to approximately half of the 12-hour value. All counts were corrected for background and for isotopic decay. Animals injected after 7 days' exposure to 5° C were injected again after 35 days' exposure, those injected after 14 days were injected again after 60 days at 5° C. Animals used after 6 months in the cold were injected only once.

RESULTS

Thyroid I^{131}. The I^{131} content of the total thyroid glands of control and experimental animals sacrificed at various intervals after I^{131} injection are shown graphically in Figure 1. In the controls of the 4 experimental groups (1, 8, 26, and 60 days) the levels of radioactivity at the 4 different times after injection were consistent. Four hours after injection, 7–10% of the dose was present in the thyroid, and 15% at approximately 12 hours. The maximum uptake occurred between 12 and 18 hours, when the radioactivity reached 20–27% of the dose. Thereafter, I^{131} content decreased rapidly to 10–15% of the dose at 24 hours. When the experimental animals had been exposed to 5° C for 1 day, the quantity of I^{131} in the thyroids at the 4-hour and 11-hour intervals did not differ significantly from that in the controls. At the 17-hour and 24-hour intervals the quantity of radioactivity was lower than that in controls.

After animals were exposed to cold for 8 days, the I^{131} content of their thyroids at the 4-hour interval was approximately twice that contained in the thyroids of the control animals but at 24 hours, the values for the experimental animals were less than those for the controls (significant at the 5% level).

After animals had been exposed to 5° C for 26 days the thyroidal I^{131} level was similar to that of the controls 4 hours after injection, while at the 12-, 21-, and 24-hour intervals, thyroidal I^{131} in the experimental animals was less than that in controls. The 60-day exposure data showed a trend similar to that for 26 days' exposure.

TABLE 2. MEAN THYROID WEIGHTS OF EXPOSED AND CONTROL ANIMALS (MG. PER 100 GM. BODY WEIGHT)

Exp. temp.	Time in cold			
	1 day	8 days	26 days	60 days
5° C	7.8±1.1* (18)‡	6.4±.2† (17)	6.1±.2† (16)	5.8±.3 (17)
26° C	7.0± .3 (19)	5.5±.3 (20)	4.6±.2 (20)	5.8±.3 (17)

* One standard deviation.
† Significant at 5% compared to the corresponding aninals at 26° C.
‡ Numbers in parentheses indicate the number of animals used.

Thyroid Weight. The thyroid weights, expressed in mg. per 100 gm. body weight, are shown in Table 2. After 1, 8 and 26 days at 5° C, the animals' thyroids weighed significantly more than those of the controls (significant at the 5% level). Thyroids from animals in the cold for 60 days showed no significant weight differences when compared to those of the controls.

Serum I^{131}. The percentage of the total serum I^{131} which was present as PBI^{131} at various intervals after injections is shown in Figure 2. There was a fairly gradual but uniform increase in conversion ratios from 4–21 hours after injection, but between 22–24 hours the percentage of serum I^{131} present as PBI^{131} increased 3–4 times. This was mainly a consequence of decreased serum inorganic I^{131} during this period. Since Nadler and Leblond (13) found that the maximum rate of secretion of newly-formed hormonal radioiodine occurred about 25 hours after the injection of the isotope the 24-hour conversion ratios are probably most significant in esti-

TABLE 3. AVERAGE SERUM LEVELS OF TOTAL I^{131} AND PBI^{131} (PER CENT OF DOSE PER 10 CC. OF SERUM)

	Serum PBI^{131}		Serum total I^{131}	
	5° C	26° C	5° C	26° C
1 day at 5° C				
4 hr.	.13	.04	3.2	4.6
12 hr.	.27	.17	1.35	2.06
17 hr.	.35	.31	1.06	2.14
24 hr.	.30	.28	0.59	0.59
8 days at 5° C				
4 hr.	.15	.05	5.7	5.4
10 hr.	.25	.06	3.6	3.1
18 hr.	.23	.26	3.5	4.2
24 hr.	.22	.23	0.34	0.51
26 days at 5° C				
4 hr.	.04	.03	4.84	2.94
12 hr.	.08	.19	0.62	2.04
21 hr.	.12	.15	1.22	1.64
24 hr.	.15	.17	0.18	0.55
60 days at 5° C				
4 hr.	.04	.03	3.16	2.94
12 hr.	.08	.09	1.04	1.85
21 hr.	.26	.19	1.49	1.68
24 hr.	.20	.08	0.26	0.24

mating thyroid hormone turnover. Twenty-four hour conversion ratios for the controls of all 4 groups were fairly consistent, although those for the 28- and 60-day groups were somewhat lower, i.e., 35% and 32%, than those for the 1- and 8-day controls, i.e., 49% and 47% respectively. This difference may have resulted from differences in age.

After animals had been exposed to 5° C for 1 day, the 24-hour ratios showed a wide range, and the mean was not significantly different from that for the controls. In the 8-day group of experimental animals, the mean of the 24-hour conversion ratios was greater than that of controls, but the difference in magnitude was not statistically significant. After exposures of 28 and 60 days, however, the animals had conversion ratios which were approximately twice those of the controls.

TABLE 4. BIOLOGICAL HALF-LIFE OF I^{131} IN EXPOSED AND CONTROL ANIMALS (DAYS)

	Time in cold				
	7 days	14 days	35 days	60 days	180 days
Animals at 5° C	1.3±0.6*†(7)‡	1.6±0.6†(5)	1.0±0.1†(4)	2.3±1.0(5)	2.3±0.5† (8)
Animals at 26° C	2.8±0.3 (9)	3.4±1.2 (4)	3.0±0.9 (8)	3.2±0.2(3)	3.5±0.4 (10)
Animals at 5° C Expressed as % of Controls	46	47	33	71	65

* One standard deviation.
† Significant at 5% when compared to the corresponding animals at 26° C.
‡ Numbers in parentheses indicate the number of animals used.

Urine I^{131}. Radioactive iodine was excreted in the urine in high concentrations (Fig. 3). However, the amount excreted varied considerably, so values for controls and experimentals could be only roughly compared. Animals exposed to 5° C for 8, 26 and 60 days excreted a larger percentage of the dose than the corresponding controls.

Biologic Decay Studies. The release of I^{131} from the thyroid as measured by the drop in radioactivity over the neck region of the intact rat is plotted in Figure 4. The biologic half-lives of radioiodine in control and experimental animals calculated from these plots are shown in Table 4. Radioiodine was released by all control animals at a fairly consistent rate; however, a slightly slower release was evident in the older animals. The biologic half-times in the control animals agree with those measured by Wolff (14), Perry (15) and Stevens and D'Angelo (8) who have reported values of 3.3, 2, and 3.5 days, respectively.

In animals exposed to 5° C for 7, 14 and 35 days, the biologic half-times were 1.3, 1.6, and 1.0 days respectively. In each case, values in the experimentals were statistically different (significant at 5% level) from those in the corresponding controls. After 60 days the experimental animals had a faster rate of release than the controls, but the difference just lacked statistical significance, possibly because the number of rats was small. Half-times in the animals which had spent 180 days at 5° C were less than those for corresponding controls, and the difference was statistically significant

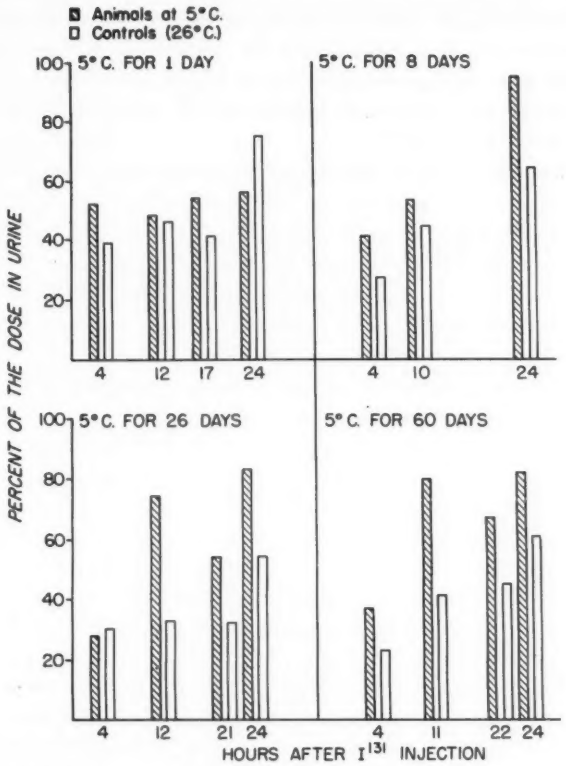

FIG. 3. Percentage of dose in urine of control rats and rats exposed to 5° C for 1, 8, 26, and 60 days, animals sacrificed at hour intervals, 4–24 hours after intraperitoneal injection of I^{131}.

(5% level). To facilitate comparison of rates of biologic decay during adaptation, the half-times expressed as percentages of the values of the corresponding controls are shown in Table 4. Although the rate of release of radioiodine was lower in animals exposed for 60 and 180 days than in animals exposed for shorter periods, it remained higher than that of controls.

DISCUSSION

The amount of I^{131} in the thyroid 4 hours after injection of rats exposed to cold for 1, 8, 26 and 60 days corresponds fairly closely with the data of Schachner *et al.*, (7) for 2-hour uptakes in that the maximum uptake occurred after 8 days' exposure and thereafter approached normal. The present experiments, however, indicated persistence of a slight but significant difference in thyroidal I^{131} of controls and experimentals after the experimentals had been 60 days in the cold.

The finding that the quantity of I^{131} in the glands of experimental animals exposed 8 days was higher than in controls at 10 and 18 hours after injection and less than in controls at 24 hours agrees with the finding of Catz and co-workers (5) who measured the I^{131} content in thyroids of rats maintained for 9 days at 3° C.

The possible existence of differences in iodine pool which might account

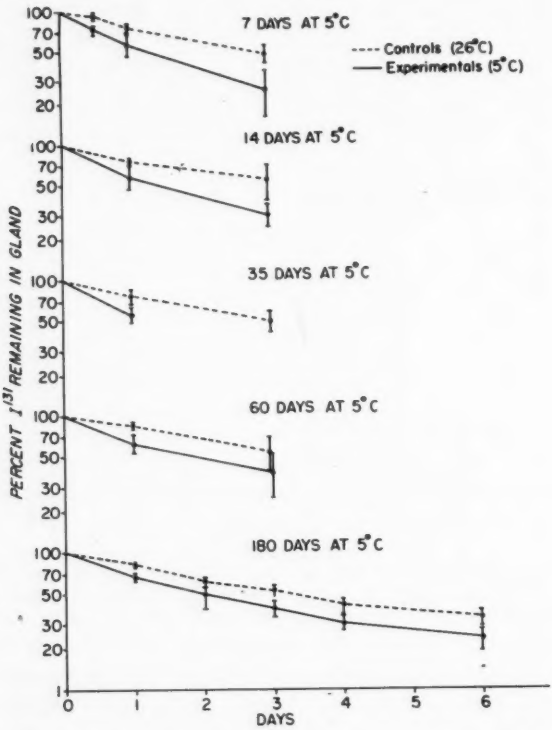

FIG. 4. Release curves for experimental animals exposed to 5° C for 7, 14, 35, 60, and 180 days and for controls. One standard deviation shown on either side of mean.

for differences in thyroidal I^{131} between the two groups cannot be overlooked. Two factors, iodine intake and iodine excretion, might operate to alter the size of the pool. It is known that the rats maintained at 5° C double their food intake (11), and thus would ingest twice as much iodine (127). If this larger intake resulted in a larger iodine pool, then the dose administered should mix with a larger quantity of iodine, and thus, the specific activity would be less in the experimentals than in the controls. However, this factor would tend to lower rather than raise the thyroid I^{131} content of the experimental animals.

If experimental animals were to excrete more I^{131} than the controls, the circulating I^{131} level and the quantity presented to the thyroid would decrease. However, the concentration of I^{131} per cubic centimeter of serum measured at the 4 intervals after injection were quite similar in experimental and control animals.

Increased weight of the thyroid gland upon exposure of animals to cold has often been reported and interpreted to represent corresponding "activity" of the gland. Since animals living at 5° C are smaller than those of similar age living at 26° C (Table 1), absolute thyroid weights are not comparable. For this reason, the thyroid weights are expressed as milligrams per 100 gm. body weight. Differences in the thyroid weights of the controls are noted in Table 2 and may be accounted for by age differences between the groups. After 8 days in the cold, the thyroid weight is increased (significant at the 5% level). Maximal increases in gland weight were found after 26 days' exposure to 5° C. After 60 days in the cold, the thyroid weights were similar to those of the controls.

The increase in the 24-hour conversion ratios evident after rats have been 8 days in the cold and the further increase after 26 days are suggestive of more rapid turnover of radioiodine. The shorter biological half-lives observed in the experimental animals are consistent with this and in agreement with the data of Stevens *et al.* (16) on biologic decay in cold-exposed (30 days) guinea pigs.

If the release of I^{131} and the 24-hour conversion ratios are valid measures of hormonal secretion, then it may be concluded that after an exposure to cold for 1 week or possibly less, turnover of thyroid hormone increases. Although after animals have been exposed for prolonged periods this rate appears to drop off slightly, it still remains higher than that of controls. This is consistent with the findings of only a slight drop in metabolism in rats exposed to cold for 60 days (11).

Comparison of the parameters of thyroid function as utilized in the present study (Table 5) indicates that during the initial period of cold exposure those which measure thyroid activity by indirect means agree quite closely with those which are more direct. However, such agreement is not evident after prolonged exposure to cold. These findings provoke speculation concerning the mechanisms controlling thyroid function.

Thyroid function is generally considered to be regulated by the serum level of thyrotropic hormone (TSH) and the latter, in turn, is thought to depend upon the circulating level of thyroid hormone. A second means for control of TSH as postulated by Uotila (17) and Brolin (12), is direct neural mechanisms. The experiments of Greer (18) suggest that TSH produced in response to decreased blood levels of thyroid hormone differs qualitatively from the TSH produced as a result of neural stimulation. He has postulated a thyroid growth component of TSH which is dependent upon the integrity of a small hypothalamic region anterior to the ventral median

nucleus, and a metabolic component which controls iodine uptake, is independent of the hypothalamus, and presumably is controlled by the circulating level of thyroid hormone.

On the basis of the thyroid's response to cold, gland weight and iodine content seem to be under similar controlling mechanisms, while secretion of the hormone would appear to be regulated separately. Possibly when rats are initially exposed to cold, the anterior pituitary is stimulated via the hypophyseal stalk, and the additional TSH (or component of TSH) so

TABLE 5. COMPARISON OF INDICES OF THYROID ACTIVITY AFTER PERIODS OF EXPOSURE TO 5° C

Indices of thyroid activity	Initial exposure (7–21 days)	Prolonged exposure (60 days or more)
Gland Weight	+	−
I^{131} Content at 4 Hours	+	−
Conversion Ratio at 24 Hours	+	+
Biologic Decay	+	+

produced affects primarily cell size and iodine uptake. As exposure continues, the increased utilization of thyroid hormone at the tissue level may cause a drop in the circulating level of thyroid hormone stimulating the production and secretion of a second TSH component. This component would presumably be primarily concerned with release of thyroid hormone and maintenance of its level in the circulating blood. The initial neural stimulus is possibly short-lived, while presumably the increased utilization of the hormone remains as long as the animals are in the cold. The possibility that the adrenal cortical hormones (stimulated during initial exposure) directly affect the thyroid response either by substituting for thyroid hormone at the metabolic level or by affecting TSH release cannot be overlooked.

SUMMARY

Thyroid function in male albino rats exposed to 5° C for 1–180 days was studied. Twenty-four hour conversion ratios $\left(\frac{\text{Serum protein-bound } I^{131}}{\text{Serum total } I^{131}}\right)$ were above control values in animals exposed for 8 days and remained higher than control values when measured in animals exposed for 60 days. Biologic decay data also indicated increased turnover of thyroid hormone during early exposure and after prolonged (180 days) exposure to 5° C. In contrast, gland weight and I^{131} content 4 hours after injection increased upon preliminary exposure, but returned to approximately control values after 60 days at 5° C indicating that these are not sensitive and consistent measures of thyroid hormone secretion.

Acknowledgments

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THE I^{131}-IRRADIATED RAT THYROID: ITS ALTERED RESPONSE TO VARIOUS STIMULI AND THE CHANGES INDUCED IN ITS IODINE METABOLISM[1]

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MANY reports dealing with the effects of I^{131} irradiation on the thyroid gland have appeared (1–13). Most of these were concerned with histological changes in the gland and with its altered response to various stimuli. The present investigation was undertaken primarily to study the effects of I^{131} irradiation on the iodide-concentrating mechanism of the thyroid and on the nature and distribution of the iodine compounds formed by the gland. At the same time we also investigated the response of the I^{131} irradiated rat thyroid to the following stimuli: 1) the feeding of propylthiouracil (PTU); 2) the injection of thyrotropic hormone (TSH); and 3) lowered environmental temperature. The radiation dosage to the thyroid was estimated from *in vivo* measurements of uptake and release of I^{131} by the gland. Histological effects of I^{131} irradiation on the thyroid are also reported here.

METHODS AND RESULTS

Long-Evans rats of both sexes, weighing 175–225 gm., and raised on Purina Laboratory Chow, were injected with varying doses of carrier-free I^{131}. Two to five weeks later their thyroids were compared with those of control rats, as described in the experiments below.

I. *Estimation of Radiation Dosage Delivered to the Thyroid Gland after Injection of I^{131} into Rats*

Method.—The uptake and release of I^{131} by the thyroid gland were followed *in vivo* with a specially designed scintillation counter. The animals were anesthetized with sodium Evipal (100 mg. per kg. body weight) during the counting procedure. Background counts were obtained from the thigh area.

Calculation.—The radiation dosage delivered to the thyroid gland was determined from the I^{131}-disappearance curves shown in Figure 1 (11).

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These curves are based on observations on 4 groups of rats, each group consisting of 4 animals. The rats received injections of 5, 25, 100, or 300 μc. Each curve in Figure 1 shows the average decrease in thyroidal I^{131} for the 4 animals in each group. The data are plotted as per cent of the injected dose against time. Plotted in this manner, the curves are corrected for I^{131} decay, and give the biological half-time of labeled iodine in the thyroid (T_B). For purposes of estimating dosage to the gland, it is necessary to obtain the effective half-time of I^{131} in the gland (T_B), which takes into consideration that I^{131} disappears from the gland by decay as well as by release. The effective half-time of I^{131} (T_E) is related to the biological half-time (T_B) as follows:

$$T_E = \frac{8\ T_B}{8 + T_B}$$

To simplify the calculation of total radiation dosage of I^{131} delivered to the rat thyroid, we have assumed that all disintegrations of I^{131} occurring within the gland are equally effective in damaging thyroid tissue. (This disregards the fact that follicles at the periphery of the gland do not receive as much ionizing radiation as the follicles at the center.) The maximum radiation dosage to the gland in rep is given by the formula $D = 16\ I_O T_E$, where I_O = initial concentration of I^{131} in μc. per gram of thyroid tissue, and T_E = effective half-time of I^{131} in the gland (11). The factor 16 is based on the use of 93 ergs per rep instead of 84 ergs per rep as formerly employed (14).

Results.—The values obtained by applying the above formulas to the curves of Figure 1 are shown in Table 1. The calculated dosages are only estimates, since many assumptions are involved in the simplified calculation. More elaborate calculations can be derived (4), but these also involve assumptions, and for the present purpose it is the relative radiation dosage delivered to the gland with different doses of I^{131} that is of interest.

The values shown in Table 1 for radiation dosages delivered to the thyroid gland are much lower than those reported by Maloof *et al.* (11) for the same injections of I^{131}. This difference can be explained by higher thyroidal uptakes of I^{131} in the experiments of Maloof *et al.*, who fed their rats a low iodine diet for 2 weeks before the radioiodine was injected.

II. *Cytological Effects of I^{131} Irradiation on the Rat Thyroid*

The rat thyroids were excised, weighed, and fixed in 10 per cent neutral formalin. They were embedded in Tissuemat, and sectioned at 6 micra. The sections were stained with hematoxylin and eosin, and duplicate sections were also stained with Masson's trichrome stain.

25 μc. of I^{131} (estimated dose—5,600 rep).—The thyroids of rats examined 30 days after injection of 25 μc. of I^{131} showed the following changes: 1) depletion and granularization of colloid in some follicles; 2) localized cellu-

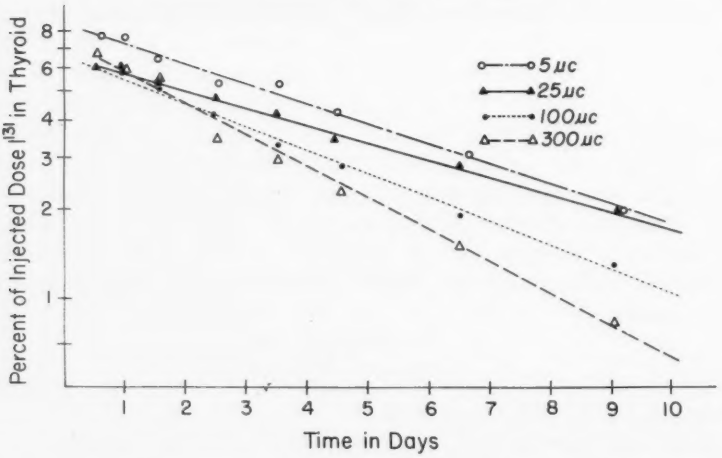

FIG. 1. Disappearance curves of I^{131} from rat thyroids as determined by *in vivo* measurements. Each point represents the average of measurements on 4 rats.

lar hypertrophy and vacuolization, especially in the smaller follicles; and 3) considerable nuclear variation, i.e., pyknotic forms and enlarged pleomorphic and hyper- or hypochromatic nuclei. An outstanding feature of these irradiated glands was the degree of variation in microscopic structure within each gland (Fig. 2B).

100 μc. of I^{131} (estimated dose—18,000 rep).—The histological appearance of rat thyroids 30 days after the injection of 100 μc. of I^{131} is shown in Figure 2C. The changes were similar to those described for the rat injected with 25 μc., but the degree of follicular damage was much more extensive, and the number of abnormal follicular cells with bizarre nuclei was greater.

300 μc. of I^{131} (estimated dose—50,000 rep).—There was extensive follicular destruction in the thyroids of rats injected with 300 μc. of I^{131} (Fig. 2D). Although groups of relatively undamaged follicles were found, these were usually small and devoid of colloid, and their epithelial cells were hypertrophied and vacuolated. Pyknotic and necrotic nuclei were common, as were also enormously enlarged and bizarre nuclei.

TABLE 1. ESTIMATED RADIATION DOSAGE DELIVERED TO THYROIDS OF RATS INJECTED WITH VARIOUS DOSES OF I^{131}

Dose	T_B (Biol. half-time)	T_E (Effective half-time)	Max. dose to gland
μc.	days	days	rep
5	4.5	2.9	1,300
25	5.0	3.1	5,600
100	3.8	2.6	18,000
300	2.8	2.1	50,000

III. *Response of the I^{131}-Irradiated Rat Thyroid to Propylthiouracil Feeding*

Procedure.—Eight groups of male rats weighing 175–200 gm. were used; each group consisted of 6 rats. Two groups were injected with 0, 25, 100 or 300 μc. of I^{131}. Fourteen days after the injection of I^{131}, 0.15% PTU was

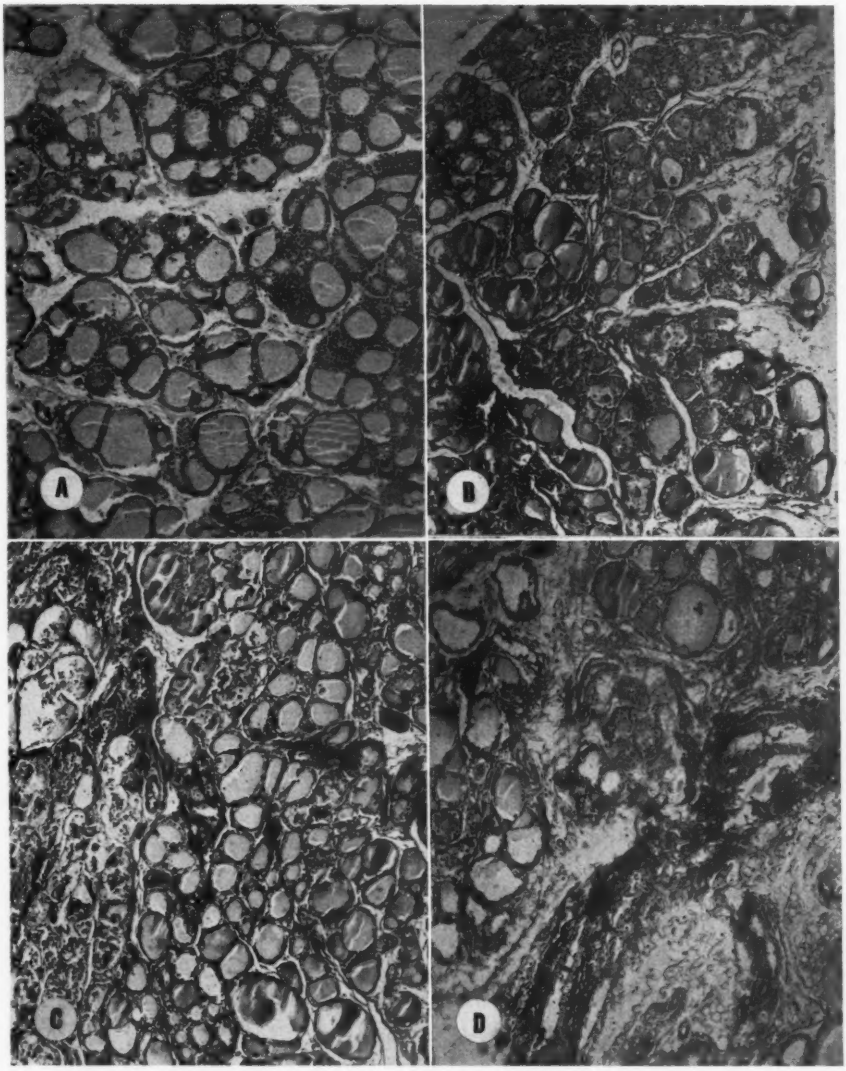

FIG. 2. Photomicrographs of rat thyroids 30 days following I^{131} injections. ×120. A, control—no I^{131}; B, 25 μc. of I^{131}; C, 100 μc. of I^{131}; D, 300 μc. of I^{131}.

added to the diet of one group of rats at each dose level. Twenty-one days later, all rats were anesthetized with Nembutal and their thyroids were excised, weighed, and fixed in 10 per cent neutral formalin for histological examination.

Results.—The mean values and standard deviations[2] of the thyroid to body weight ratios of each group of rats are presented graphically in Figure 3. The irradiated rat thyroids did not respond to propylthiouracil to the

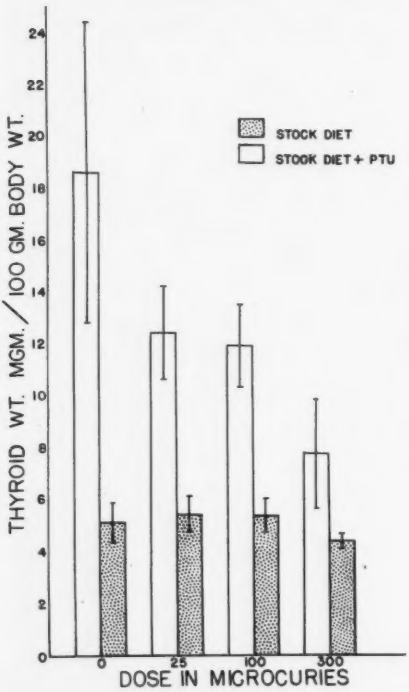

FIG. 3. Weight response of I^{131}-irradiated rat thyroid glands to the feeding of a diet containing 0.15 per cent PTU. The rats were placed on the diet 14 days after receiving I^{131}, and were sacrificed 21 days later. Each bar represents the average of measurements on 6 rats. The vertical lines show the standard deviation of the mean.

same extent as did the nonirradiated controls. The lowest radiation dose at which a significant decrease in response was obtained was 5,600 rep (25 μc.). The decrease in response observed in a separate experiment in rats injected with 10 μc. was not statistically significant.

It is interesting that the growth response of the gland to PTU feeding was not completely abolished even in rats that received the largest dose of I^{131} (300 μc. or 50,000 rep). Histological examination of such glands (Fig. 2D) revealed extremely large areas of follicular destruction. Apparently the deformed but intact follicles retained some functional activity and were able to respond to the PTU feeding.

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[2] S. D. $= \sqrt{\frac{\Sigma d^2}{n-1}}$

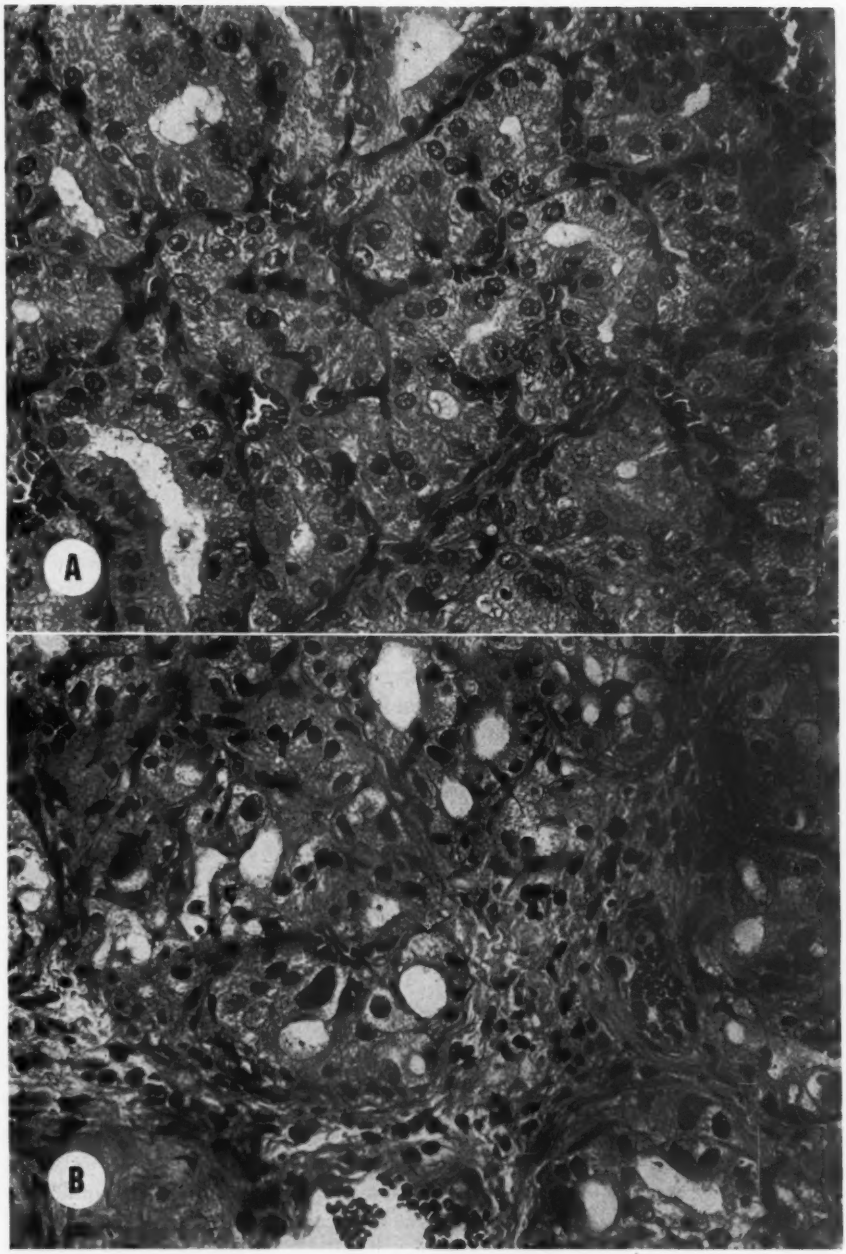

FIG. 4. Photomicrographs of thyroids of rats fed a diet containing 0.15 per cent PTU for 21 days. A, not previously injected with I^{131}; B, injected with 100 μc. of I^{131} 14 days before being placed on the PTU diet. ×480.

I^{131}-irradiated thyroids, exposed to the stimulation of PTU feeding, showed cytological changes that were different in certain important respects from the usual effects of PTU feeding (Fig. 4). The major differences were: 1) many pyknotic and bizarre nuclear forms were present in the irradiated gland after PTU feeding. 2) Cellular hypertrophy was less pronounced in the irradiated gland than in the non-irradiated gland after PTU feeding.

IV. *Response of the I^{131}-Irradiated Rat Thyroid to Injections of Thyrotropic Hormone*

Procedure.—Eight groups of rats (175–200 gm.) were used in this experiment. Each rat was injected with either 0, 25, 100, or 300 μc. of I^{131}, and 30 days later some of the groups received injections of TSH (Parke, Davis and Co., preparation Rx 099931, not assayed). Each rat was injected with 5 mg. twice daily for 4 successive days, and with 5 mg. on the fifth day; it was sacrificed 2 hours after the last injection. Control rats received saline injections at corresponding times.

Results.—The growth response of thyroid glands of normal and I^{131}-treated rats to injected TSH is shown in Figure 5. The thyroids of the rats injected with 25 μc. showed a definite impairment in their ability to respond to the injected hormone. In the groups injected with 100 or 300 μc. the thyroids failed almost completely to respond to this stimulus.

Histological examination of I^{131}-irradiated thyroids exposed to the added stimulus of TSH injections revealed that, as in the case of the PTU fed rats, there was an increase in the number of pyknotic and bizarre nuclei. The injection of TSH in addition to the radiation was more effective in producing these nuclear changes than was I^{131} irradiation alone.

V. *Response of the I^{131}-Irradiated Rat Thyroid to Cold*

It is well known that thyroids of rats subjected to a sudden lowering of environmental temperature undergo hypertrophy and hyperplasia (15–17). The effect of I^{131} irradiation on the ability of thyroids to respond to this stimulus was also studied.

Procedure.—Thirty-two male rats, weighing from 175–200 gm., were divided into 4 equal groups, and each rat received either 0, 25, 100, or 300 μc. of I^{131}. Thirty days later, the rats were shaved with an electric clipper. (It was found that shaving the animals before exposure to cold yielded more consistent thyroid enlargement.) The animals were then placed in a cold room (4° C) for 60 hours. At the end of 60 hours the rats were anesthetized with Nembutal, and the thyroids were removed, weighed, and fixed in 10% formalin. The control rats injected with 0, 25, 100, or 300 μc. of I^{131} were the same as those used for the TSH experiment (section IV) as both experiments were run simultaneously.

Results.—Exposure to cold resulted in definite enlargement of the thy-

roid glands (Fig. 6). A small (but not statistically significant) impairment in the ability of the thyroids to respond to cold was noted even in rats that had been injected with 25 μc. of I^{131}. Injections of 100 or 300 μc. significantly decreased the growth response to cold.

Histological examination of I^{131}-irradiated thyroids of rats exposed to cold revealed changes similar to those described above for I^{131}-irradiated glands injected with TSH (section IV).

VI. *Effect of I^{131} Irradiation on Iodide-concentrating Capacity of Rat Thyroids*

Procedure.—The iodide-concentrating capacity of rat thyroids (T/S ratio) was measured in the presence of PTU, essentially as outlined by Vanderlaan and Greer (18). Thirty-two male rats (185–200 gm.) were divided into 4 equal groups, and each rat was injected with either 0, 25, 100, or 300 μc. of I^{131}. Thirty days later, the animals were injected intraperitoneally with 20 mg. of PTU, and exactly 1 hour later with 100 μc. of I^{131}. Exactly 1 hour after this last I^{131} injection the rats were anesthetized with Nembutal, blood was withdrawn by cardiac puncture, and the thyroids were removed and weighed. Since an appreciable amount of I^{131} from the first injection remained in the gland, it was necessary to fractionate the

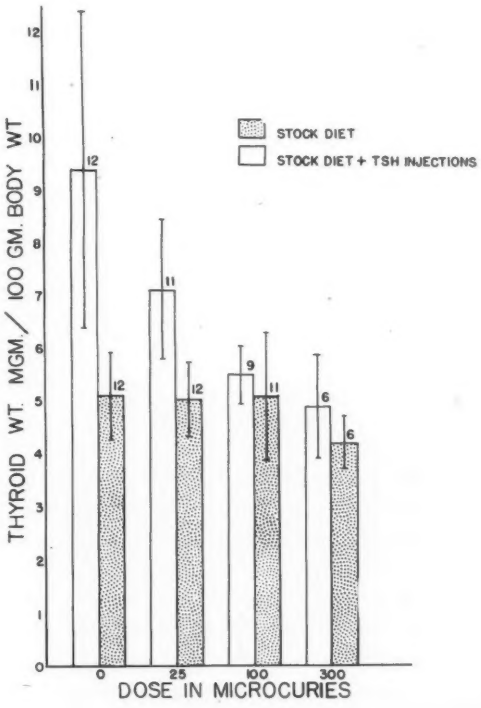

FIG. 5. Weight response of I^{131}-irradiated rat thyroids to injections of TSH. Thirty days after they were injected with I^{131}, the rats received 5 mg. of TSH twice daily for 4 days and one injection on the fifth day. They were sacrificed 2 hours after the last injection. The numbers which appear directly above the bars indicate the number of animals in the various groups. The vertical lines show the standard deviation of the mean.

thyroidal I^{131} to determine that portion which was newly taken up by the gland. The residual iodine was shown, in preliminary experiments, to be almost entirely insoluble in trichloroacetic acid. In order to measure newly concentrated, inorganic-I^{131}, the thyroids were homogenized with 1 ml. of cold 20% trichloroacetic acid. The mixture was centrifuged, and the residue was washed once with 3 ml. of dilute trichloroacetic acid. The supernatant solution and washing were combined, made to required volume, and an aliquot was counted. A portion of the plasma was also counted at the same time. The T/S ratio was calculated as

$$\frac{\text{TCA-soluble } I^{131} \text{ per 100 mg. of thyroid}}{\text{Total } I^{131} \text{ per 100 } \mu\text{l. of plasma}}.$$

Results.—The T/S ratios are shown in Figure 7. The mean value and standard deviation for the group that received no I^{131} was 102 ± 27. In the group injected with 25 μc., the mean value was 90 ± 15, a value not significantly different from that for the control group. In the 100-μc. group however, a barely significant ($.02 < P < .05$) decrease from the control T/S ratio was observed (71 ± 13.5). This decrease became very marked and highly significant for the 300-μc. group (58 ± 13.0).

The results of this experiment suggest that the iodide-concentrating

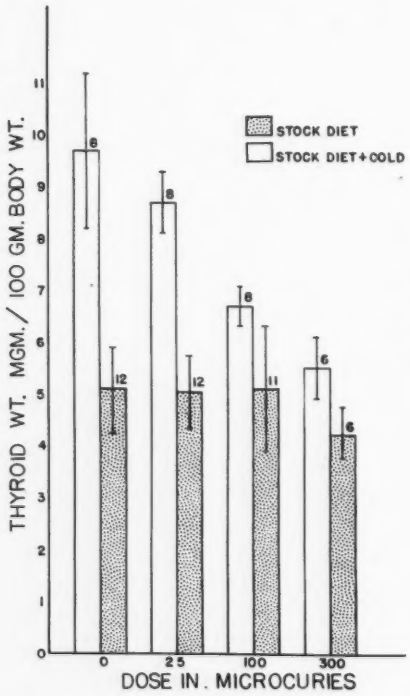

FIG. 6. Weight response of I^{131}-irradiated rat thyroids to exposure to cold. Thirty days after they were injected with I^{131}, the rats were placed in a cold room at 4° C for 60 hours. The numbers which appear directly above the bars indicate the number of animals in the various groups. The vertical lines show the standard deviation of the mean.

capacity of the thyroid gland is not so sensitive to radiation as is its ability to respond to PTU feeding or to TSH injections. When the radiation dose is high enough, however, there is marked impairment in the ability of the thyroid to concentrate iodide.

VII. *Effect of I^{131} Irradiation on Organic-I^{131} Formation by the Thyroid Gland*

Procedure.—A method for separating the I^{131} compounds in the thyroid gland by filter paper chromatography has been reported elsewhere (19, 20). Chromatography was applied to unhydrolyzed thyroids as well as to enzymatic digests of the glands. The former procedure yields information

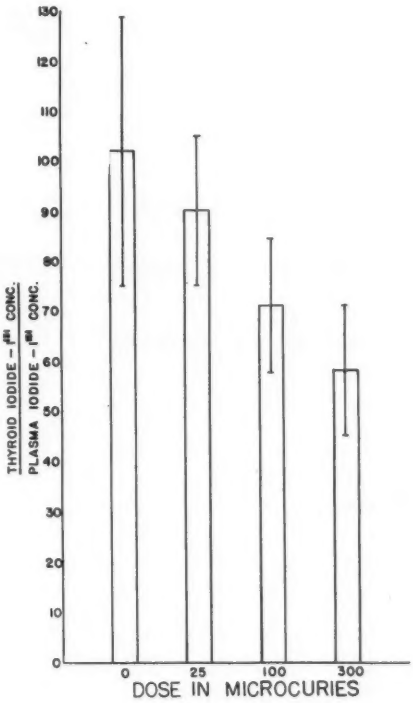

FIG. 7. Effect of I^{131}-irradiation on the iodide-concentrating capacity of the rat thyroid. The rats were injected with I^{131} thirty days before the T/S ratios were determined. Each bar represents the average of measurements on 8 rats. The vertical lines show the standard deviation of the mean.

about the free iodinated amino acids which may be present in addition to thyroglobulin and iodide. The enzymatic digest yields information on the distribution of I^{131} among the various iodinated amino acids that are part of the thyroglobulin molecule.

Fifteen female rats (165–180 gm.) were divided into 5 equal groups. Each rat was injected with either 0, 5, 25, 100, or 300 μc. of I^{131}. At the end of 33 days, each rat received a second intraperitoneal injection of approximately 35 μc. of I^{131}, and 23 hours later the rats were sacrificed. The thy-

roids of the rats in each group were weighed, pooled, and then homogenized with 0.75 ml. of bicarbonate-Ringers solution containing 25 μl. of 0.0078M thiouracil. Aliquots of the homogenate were diluted and counted to determine the average uptake of I^{131} in the thyroids of the various groups. Twenty μl. of each homogenate were analyzed by paper chromatography, both before and after enzymatic digestion. For enzymatic digestion, 0.5 ml. of homogenate was incubated with 10 mg. of pancreatin for 16 hours at 37C°. After the filter paper chromatograms had been exposed for radio-

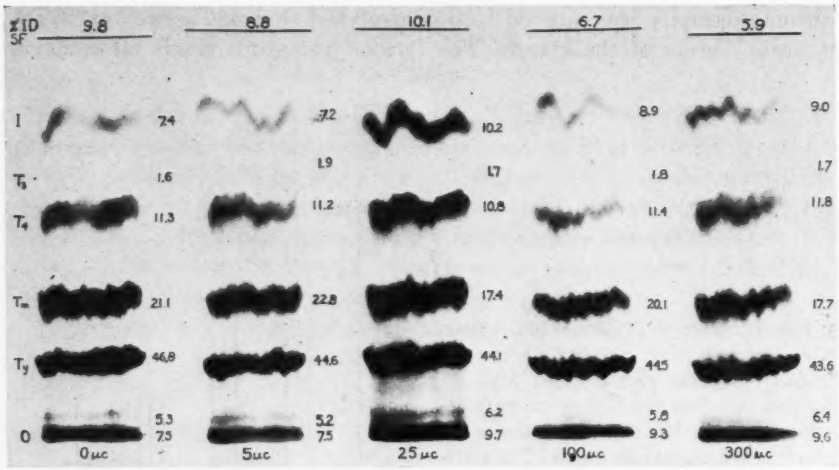

FIG. 8. Radioautographs of chromatograms prepared from pancreatin digests of rat thyroids that had been exposed to I^{131} irradiation. Thirty days after they had been injected with I^{131}, the rats received a second (labeling) injection of I^{131}, and their thyroids were removed 24 hours later. There were 3 rats at each dosage level, and the thyroids of the animals in each group were pooled for chromatographic analysis and measurement of uptake. The numbers at the base of the radioautographs indicate the initial dose of I^{131}, and the numbers above the radioautographs indicate the average uptake of the second (labeling) dose. The numbers alongside the bands of the radioautographs indicate the percentage of the I^{131} on the chromatogram present in the various components. O, origin; Ty, diiodotyrosine; Tm, monoiodotyrosine; T_4, thyroxine; T_3, triiodothyronine; I, iodide; S.F., solvent front. Solvent: collidine-water-ammonia.

autography, the various I^{131} bands on the chromatograms were cut out, placed in vials, and counted in a well-type scintillation counter.

Results.—Radioautographs of chromatograms of thyroid digests prepared from the different groups of rats are shown in Figure 8. The numbers to the right of the various bands indicate the percentages of the total I^{131} on the chromatogram found in various components. The single number at the top of each figure is the average per cent uptake of the injected I^{131} in the thyroids of that group. It is evident from Figure 8 that there is very

little difference between the 0-μc. group and the 300-μc. group with regard to the manner in which the I^{131} is distributed among the various compounds in the thyroid. This applies not only to the formation of the intermediate iodotyrosines, but also to the formation of thyroxine and triiodothyronine. Chromatographic analysis of the unhydrolyzed tissue also revealed no differences between normal and irradiated glands. No abnormal I^{131}-components were detected in the thyroids of the irradiated rats either before or after hydrolysis. A second experiment, identical with that described above except that the thyroids were examined 22 days after the first I^{131} injection, yielded similar results.

DISCUSSION

The sensitivity of the growth response of the thyroid to radiation has been noted by previous investigators. Skanse (2) found that after I^{131} irradiation the thyroids of 5-day-old cockerels did not grow normally and failed to respond normally to thiouracil feeding or to TSH injection. Maloof, Dobyns, and Vickery (11) found that rat thyroids after I^{131} irradiation, did not respond normally to the feeding of a low-iodine diet or a thiouracil-containing diet. Doniach (8), too, found that I^{131}-irradiated rat thyroids do not respond normally to the feeding of thiouracil.

We have also observed that the growth response of the thyroid is readily altered by I^{131} irradiation. In addition, our findings lead us to conclude that the ability of the thyroid to metabolize iodine is not so sensitive to radiation damage as is its ability to undergo hypertrophy. In our experiments, neither the iodide-concentrating capacity of the thyroid nor its conversion of I^{131} to thyroxine were affected by exposure to 5,600 rep, a dose that definitely reduced the ability of the thyroid to undergo hypertrophy in response to PTU feeding or TSH injections.

While the present investigation was in progress, two papers appeared dealing with the effect of I^{131} irradiation on the iodide-concentrating capacity of the thyroid. Doniach and Logothetopoulos (9) found no appreciable change in the thyroid to plasma iodide ratios of rats injected with 30 μc. of I^{131} 3 months previously. The radiation dosage in this case, although not reported by these workers, must have been considerably higher than that received by thyroids of our rats injected with 25 μc. of I^{131}, since the peak uptakes of I^{131} were about twice as high in their experiments as in ours. In a preliminary note, Maloof and Rapp (21) reported an increase in the T/S ratios of rats that received 5 μc. of I^{131} one month before. After 4 months, however, the T/S ratios dropped to $\frac{1}{2}$ to $\frac{1}{3}$ of the control value. Although an increase in T/S ratio might be expected from the slight cellular hypertrophy in the thyroids of rats exposed to relatively small doses of radiation, neither our results nor those of Doniach and Logothetopoulos (9) confirm the findings of Maloof and Rapp (21) on this point.

Maloof and Rapp also indicated in their preliminary note (21) that the thyroid's ability to synthesize hormone is more sensitive to I^{131} irradiation than is its ability to concentrate iodide. This conclusion was based on their finding a lowered serum protein-bound iodine (1 month after I^{131} injection) when they found the T/S ratio to be higher than normal. In the experiments reported here, however, we found a lowering of the T/S ratio after I^{131} injection, contrary to the findings of Maloof and Rapp. Our results show that, after the thyroid gland is exposed to I^{131} irradiation sufficient to lower both the T/S ratio and the total uptake of I^{131}, it is still able to convert to organic form in a normal manner all the I^{131} that it concentrates from the circulation. It should be noted that these findings do not necessarily imply that organic iodine formation is unaffected by I^{131} irradiation since the *capacity* of the thyroid to form organic iodine may have been reduced in the same proportion as the T/S ratio. This reduction in the iodine-concentrating capacity may not have affected the distribution of I^{131} within the gland, and could have been missed in our experiments. It is clear, however, that under the experimental conditions used here, organic iodine formation is not *more* sensitive to I^{131} irradiation than is the iodide-concentrating mechanism of the thyroid. The possibility that the former is less sensitive to radiation effects than is the iodide-concentrating mechanism is not excluded by the results obtained here.

Doniach and Logothetopoulos (9) concluded that I^{131} irradiation acts primarily upon cellular division rather than on cellular hypertrophy. They counted mitotic figures in I^{131}-irradiated rat thyroids that were stimulated by goitrogen feeding at various intervals after the I^{131} administration. The number of mitoses was greatly reduced in I^{131} irradiated glands. Maloof *et al.* (11) also suggested that moderate I^{131}-irradiation impaired cellular division but not cellular hypertrophy. The cytological findings in our experiments do not seem to confirm this view. The cellular hypertrophy induced by the feeding of PTU to our rats appears significantly less in irradiated than in nonirradiated thyroids (Fig. 4), although it is difficult to be certain of this without cell height measurements.

Nuclear changes induced by I^{131} irradiation of thyroids have been noted earlier (9, 11, 22). In our experiments also, the nuclei were particularly sensitive to radiation. Pyknosis and occasional enlargement of nuclei were seen in the thyroids of rats that received only 25 μc. of I^{131}. These glands received an estimated dose of only 5,600 rep., to our knowledge the smallest dosage yet reported to have definite histological effects on thyroid tissue. The occurrence of bizarre nuclei in I^{131}-irradiated thyroids was greatly augmented in our experiment, as in those of Maloof *et al.* (11) and of Doniach and Logothetopoulos (9), by stimulating the thyroids in various ways after the radiation exposure. All of the stimulatory procedures presumably had in common the elevation of the blood level of TSH.

In a previous paper from this laboratory (6), it was reported that histo-

logical effects were not observed in the thyroids of rats one month after they had received 18 μc. of I^{131} (estimated dose to a point at the center of the gland—over 12,000 rep.). These rats had been raised and maintained on a low-iodine diet. In the present study, definite histological effects were observed when lower doses of I^{131} were applied to the gland. We have no explanation for this discrepancy.

SUMMARY

1. Rats were injected with carrier-free doses of I^{131} ranging from 5 to 300 μc. Radiation dosage to the thyroid gland was calculated from *in vivo* uptake and disappearance of I^{131} from the glands.

2. Two to 5 weeks after the I^{131} injection, the irradiated thyroids were compared with control thyroids with respect to: a) iodide-concentrating capacity; (b) synthesis of organic I^{131} compounds; c) response to the feeding of propylthiouracil; d) response to cold; e) response to TSH injection; and f) cytology.

3. The most sensitive index of radiation damage to the thyroid gland was diminished growth response to stimuli that normally produce hypertrophy and hyperplasia. Cytological changes were also noticeable at relatively low radiation doses.

4. A reduction in the iodide-concentrating mechanism (T/S ratio) of the thyroid was observed only after relatively large doses of radiation. The ability of the thyroid gland to form I^{131}-thyroxine and other organic I^{131}-compounds was possibly as sensitive—but certainly not more sensitive—to radiation damage than was the iodide-concentrating capacity. Even after the thyroids had been exposed to the highest radiation dosage employed in the present study (50,000 rep.), the *distribution* of I^{131} among the various iodine compounds of the gland was indistinguishable from normal.

5. It appears, from the data obtained here, that those thyroid processes concerned with growth are more sensitive to radiation than are those concerned with iodine metabolism.

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CHANGES IN EXTRACELLULAR FLUID LEVELS OF CALCIUM, PHOSPHATE AND CITRATE IONS IN NEPHRECTOMIZED RATS FOLLOWING PARATHYROIDECTOMY[1]

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MUCH of the recent interest in parathyroid physiology has pertained to its extrarenal function. Numerous reports have shown that serum calcium changes could be produced in nephrectomized animals both by parathyroidectomy and the administration of parathyroid extract (1–4). Among the most impressive of these studies was the report by Grollman (5) in which he was able to control the serum calcium level of nephrectomized-parathyroidectomized dogs maintained by peritoneal lavage.

The extrarenal action of the parathyroids has also been emphasized by Munson and his collaborators (6) who have reported on the variations in the ratio of various parathyroid extracts in their ability to raise serum calcium levels and to increase the excretion of phosphate by the kidney. Also, in regard to extrarenal influences, it has been observed in several different species that parallel elevations of serum citric acid and calcium levels occurred following parathyroid extract administration (7). While the ratio of calcium to citric acid in the serum varied considerably under experimental conditions, the fact that the concentrations of the two ions always changed in the same direction suggests very strongly that bone citric acid is in some way connected to the effect of the parathyroids on bone metabolism.

In contrast to these extrarenal studies, our laboratory in recent reports (8, 9) has emphasized the importance of the control by the parathyroids of renal excretion of both calcium and phosphate. These reports have suggested that, at least for the rat, the hormone of the parathyroids is able to raise the renal threshold for calcium while lowering that for phosphate. These kidney changes could be demonstrated within one hour after the administration of parathyroid extract or after parathyroidectomy.

The studies reported below were instigated in an attempt to compare changes in renal excretion of calcium and phosphate to extrarenal changes both as to the time element involved and the manner in which calcium and phosphate ions were affected. The experiments were carried out using nephrectomized rats maintained by peritoneal lavage and the results show that the extrarenal influences of the parathyroid hormone are realized in equally

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TABLE 1. COMPOSITION OF RINSING FLUID FOR PERITONEAL LAVAGE

Contents per liter of solution		Calculated content in mg./100 ml.	
KCL	0.1225 gm.		
$MgCl_2 \cdot 6H_2O$	0.035 gm.	Sodium	348.0
NaCl	6.75 gm.	Potassium	5.9
$NaHCO_3$	2.19 gm.	Dextrose	3000.0
NaOH	0.24 gm.	Calcium	void
Lactic Acid (USP—85%)	0.675 ml.	Phosphate	void
Dextrose	30.0 gm.		
Distilled Water	to 1000 ml.		

as short a time as are the renal effects, and that the changes produced in the extracellular concentrations of calcium, phosphate and citrate ions are all of approximately equal magnitude and in the same direction. These studies are in support of the thesis that the parathyroids maintain a constant serum level for calcium by making available calcium and phosphate from the bone while simultaneously raising the renal threshold for calcium and lowering that for phosphate.

MATERIAL AND METHODS

Male Sprague-Dawley rats weighing between 250 and 325 grams were used in these experiments. However, for any one experiment, the weight range was restricted to 25 grams. For most of the experiments the animals were maintained on standard purina diet. Two additional experiments, however, were run in which the animals were maintained three days prior to use on a calcium free diet in order to rule out the possibility that the results obtained could be due to changes in intestinal absorption of calcium. In preliminary experiments, nephrectomy was performed using dorso-lateral incisions. In all other experiments the animals were nephrectomized through a ventral midline incision. At the same time a peritoneal plug made from the specifications of Kolff and Page (10) was stitched into the incision.

Peritoneal lavage (three times daily) was accomplished by using a davol catheter attached to a 50 ml. syringe. Thirty ml. of rinsing fluid (composition in Table 1) were placed in the peritoneal cavity and after one hour removed by reinsertion of the catheter. For protection against infection, penicillin and streptomycin were inserted through the peritoneal plug at the conclusion of the operation and daily thereafter. The parathyroids were removed individually. Controls for these groups were sham operated. All operations were done under ether anesthesia. Parathyroid extract[2] was diluted with an acetate buffer with no change in pH. Control animals were injected with acetate buffer alone. These solutions were injected subcutaneously.

Determinations of calcium, potassium and sodium were done using a Beckman flame spectrophotometer. Citric acid determinations were made by the method of Pucher *et al.* (11); phosphate determinations by the Gomori method (12).

RESULTS

The data obtained in these studies have been summarized in the four series of experiments given below:

1. *The Effect of small Amounts of Parathyroid Extract in Parathyroidec-*

[2] Lilly's Parathyroid Extract.

TABLE 2. EFFECT OF PARATHYROID EXTRACT (1.0 I.U./HR. FOR 6 HOURS) GIVEN TO RATS NEPHRECTOMIZED 3 HOURS AFTER PARATHYROIDECTOMY
(All values given as mg./100 ml. with standard error)

Groups	No. of animals	Serum calcium	Serum PO_4 in mg. P
1. Normal Animals	40	10.7 ± .17	9.0 ± .28
2. Controls—Nephrectomized for 6 hours	4	15.1 ± .5	13.9 ± .12
3. Controls—Parathyroidectomized for 9 hours	22	7.0 ± .24	10.6 ± .33
4. Controls—Parathyroidectomized for 9 hours; Nephrectomized for 6 hours	6	9.4 ± .40	13.4 ± .34
5. Same as 4 with Parathyroid Extract started following Nephrectomy	7	11.2 ± .7	15.5 ± .37

tomized-Nephrectomized rats: This series of experiments was done to show, first, the small amount of extract needed to produce changes in the parathyroidectomized animal, and, second, that in nephrectomized rats parathyroid extract produced parallel changes in calcium and phosphate concentrations of serum. The data are summarized in Table 2. For this work the rats were first parathyroidectomized, and three hours later the kidneys removed. Immediately after nephrectomy, parathyroid extract was administered hourly for six hours at which time the animals were sacrificed. A total of 6 I.U. of parathyroid extract administered over a six hour period raised both the serum calcium and serum phosphate approximately 2 mg./100 ml. above their parathyroidectomized-nephrectomized controls.

2. *Peritoneal Wash Analysis in Control Rats Following Nephrectomy:* This group of experiments was run as a control series to establish the normal changes after nephrectomy in the primary fluid electrolytes as well as of those specific ions under study. The data are summarized in Table 3. It is of interest that despite the frequency of the lavage with a solution low in potassium and void in phosphorus, the concentration of these two ions in the wash gradually rose throughout the experimental period. Another point to be noted is the high citric acid concentration found immediately following nephrectomy and the return to a constant level within twenty-four hours. The last point to be emphasized is the consistency in the concentration of calcium in the wash, except in that removed 12 hours after nephrectomy, at which time there was a significant drop.

TABLE 3. PERITONEAL WASH ANALYSIS IN CONTROL RATS FOLLOWING NEPHRECTOMY
(All values given as mg./100 ml. with standard error)

Hours after nephrectomy	No. of animals	Na	K	Citric acid	P	Ca
Wash H_2O Before Use	10 (samples)	329 ± 5.0	6.4 ± .2	—	—	—
4	24	295 ± 5.0	17.1 ± .3	19.2 ± .4	5.3 ± .3	6.9 ± .2
12	20	266 ± 3.0	16.9 ± .5	4.5 ± .2	7.2 ± .4	5.0 ± .1
24	43	283 ± 3.0	21.2 ± .3	1.4 ± .03	9.5 ± .1	6.3 ± .1
48	14	278 ± 5.0	25.1 ± .7	1.4 ± .06	13.3 ± .6	6.6 ± .1

3. *Peritoneal Wash Analysis in Nephrectomized Rats Following Parathyroidectomy:* In this series of experiments, parathyroidectomy was performed either 24 or 48 hours after nephrectomy. While the results in all cases were similar, because of the differences in the phosphate levels on these days (see Table 3), only the experiments involving parathyroidectomy 24 hours after nephrectomy are reported. These data are summarized in Table 4. Twenty-four hours after parathyroidectomy, part of this group of animals was given parathyroid extract in six hourly injections. Of special

TABLE 4. PERITONEAL WASH ANALYSIS IN NEPHRECTOMIZED RATS FOLLOWING PARATHYROIDECTOMY
(All values given as mg./100 ml. with standard error)

Experimental condition	Groups	No. of animals	P	Ca	Citric acid
1. 24 Hours Post-Nephx.[1] Pre-PTX[2]	All Animals	24	8.8 ± .4	6.1 ± .1	1.4 ± .06
2. 28 Hours Post-Nephx.	Controls	19	10.9 ± .6	6.5 ± .2	1.5 ± .04
4 Hrs Post-PTX	PTX	22	8.3 ± .4	3.9 ± .2	1.0 ± .03
3. 48 Hours Post-Nephx.	Controls	6	12.4 ± .9	6.8 ± .1	1.6 ± .02
24 Hours Post-PTX	PTX	11	9.5 ± .6	3.7 ± .2	1.2 ± .05
4. 54 Hours Post-Nephx.	Controls	5	9.9 ± .1	5.6 ± .1	1.6 ± .2
30 Hours Post-PTX	PTX	5	7.5 ± .7	3.7 ± .3	1.0 ± .05
6 Hours PTH (l.i.u./hr.)	PTH[3]	7	8.7 ± .9	5.4 ± .3	1.6 ± .04
5. Serum values of blood taken from animals at time of #4 above	Controls	8	12.8 ± .76	9.4 ± .34	—
	PTX	5	9.3 ± .20	7.1 ± .35	—
	PTH	4	10.5 ± .10	9.0 ± .20	—

[1] Nephrectomized.
[2] Parathyroidectomized.
[3] Administered Parathyroid Extract.

interest is the corresponding fall in wash concentration of phosphate, calcium and citric acid after parathyroidectomy and the immediate increase produced by the injection of as little as 6 I.U. of parathyroid extract. However, the calcium ion concentration, though lower, remained throughout the experiment as constant for the parathyroidectomized rat as was noted for the control animals. The serum values of samples taken at the conclusion of the experiment are given to show that the concentration of the wash is a true reflection of the serum level of these electrolytes.

4. *Peritoneal Wash Analysis in Nephrectomized Rats 1–3 Hours after Parathyroidectomy:* This series of experiments was performed to determine whether or not changes following parathyroidectomy in the nephrectomized animals could be detected as early as for the renal changes reported from this laboratory (8). The data are summarized in Table 5. For these experiments, the rats were parathyroidectomized 24 hours after nephrectomy. Thirty minutes later wash water was placed in the peritoneal cavity and allowed to remain only for an additional half hour. At the conclusion of this period, the first wash was removed and a second wash inserted and

allowed to equilibrate for one hour. The rats were killed two hours after parathyroidectomy. A significant drop in the calcium concentration had occurred by the end of the first hour, and during the second hour, values for calcium, phosphate and citric acid of parathyroidectomized rats were statistically lower than their controls. Serum analyses of blood removed two hours after parathyroidectomy showed the wash concentration to be true reflections of serum levels.

TABLE 5. PERITONEAL WASH ANALYSIS AND SERUM VALUES 24 HOURS POST-NEPHRECTOMY, 1–2 HOURS POST-PARATHYROIDECTOMY
(All values given as mg./100 ml. with standard error)

Experimental condition	Groups	No. of animals	P	Ca	Citric acid
				Wash	
1. 24 Hrs. Post-Nephx.[1] Pre-PTX[2]	All Animals	11	11.8 ± .2	6.7 ± .1	1.3 ± .03
2. 24 Hrs. Post-Nephx. 30–60 min. PTX	Controls	5	7.7 ± .4	4.2 ± .2	.7 ± .05
	PTX	6	7.2 ± .5	3.5 ± .1	.8 ± .03
3. 24 Hrs. Post-Nephx. 1–2 Hrs. PTX	Controls	5	9.0 ± .4	5.2 ± .2	.9 ± .03
	PTX	6	7.4 ± .1	3.5 ± .1	.76 ± .12
				Serum	
4. Serum Values #3 above	Controls	5	10.7 ± .5	9.7 ± .4	—
	PTX	6	8.8 ± .1	6.1 ± .1	—

[1] Nephrectomized.
[2] Parathyroidectomized.

DISCUSSION

As the result of the experiments reported here, there are several observations that appear pertinent to the physiological actions of the parathyroid hormone. The first of these concerns the changes in calcium and citric acid levels of the serum during the first 24 hours after nephrectomy. Elliott and Freeman (13) have reported a marked rise in both calcium and citric acid levels immediately after nephrectomy, followed by a drop to normal or subnormal concentrations within 24 hours. Our data, as presented in Tables 2 and 3, appear to confirm their findings, bearing out their contention that nephrectomy is followed by compensatory changes in parathyroid secretion until an equilibrium is again reached. Because of the results, parathyroidectomy in the studies reported here was always delayed until at least 24 hours after nephrectomy.

The second observation concerns the role of the parathyroids in maintenance of a constant calcium ion concentration by influencing the equilibrium between bone and extracellular fluid. It will be noted that despite the fact that the peritoneal cavity was always washed three times a day with a calcium-free wash, the calcium levels of the wash fluid removed from the peritoneal cavity after the equilibrium period was constant for any given experimental situation. In rats with intact parathyroids, the calcium con-

centration was maintained at a level between 6 and 6.8 mg. per 100 ml. of fluid, while after parathyroidectomy this was reduced to between 3.5 and 4 mg. per 100 ml. and remained constant within those limits. This is a convincing support of the feed-back mechanism of parathyroid action on bone recently reported by McLean and Urist (14), indicating that the function of the hormone in regard to bone is to increase the amount of labile bone material which is in equilibrium with the calcium ions of the extracellular compartments.

The third observation is that changes in fluid calcium concentrations in nephrectomized animals in response to parathyroid changes are always accompanied by changes in the same direction of both phosphate and citric acid ions. Changes in phosphate ion concentrations paralleling those in calcium are what would be expected in any effect on bone salts. The emphasis is made here for it has frequently been the tendency to refer only to removal of calcium from bone as a response to parathyroid extract without cognizance of the fact that corresponding amounts of phosphate are also removed. Citric acid changes paralleling those of calcium have been reported recently by L'Heureux and Roth (15) and Elliott and Freeman (13). These studies further emphasize the close relationship between citric acid, calcium and phosphate metabolism of bone. They do not give any indication, however, as to whether the citric acid changes are the result or cause of the changes seen in calcium and phosphate concentrations.

The last observation pertains to the time lapse before parathyroidectomy changes can be detected in nephrectomized animals. Previous work from this laboratory has emphasized that the kidney changes in phosphate and calcium excretion can be measured within one hour after removal of the parathyroids from the rat. The present studies show that the bone changes in nephrectomized rats can also be detected within one hour after parathyroidectomy. The rapid changes in the bone-fluid equilibrium for calcium and phosphate produced by parathyroidectomy or parathyroid extract administration would lead one to suspect that the bone changes are either of extracellular origin or are mediated through existing bone cells and are not dependent upon the production of detectable histological changes in the cellular components. This point, however, seems worthy of additional study.

SUMMARY

The ability of the parathyroids to maintain serum calcium levels in the absence of the kidneys was studied in rats by the use of a peritoneal plug which permitted continual peritoneal lavage. Since nephrectomy appeared to produce transitory changes in parathyroid function, parathyroidectomy was not performed until at least 24 hours after kidney removal. Parathyroid extract, where administered, was diluted down with acetate buffer to maintain the 3.7 pH of the extract. Controls were injected with the

buffer alone. The wash solution consisted of an isotonic saline containing 3% glucose, but void in both phosphate and calcium ions. The peritoneal cavity was washed three times daily with 30 ml. of the solution, each wash remaining in the animal for one hour. Analyses were made of the wash after removal from the animal. The following observations were noted: In the control animals, the calcium concentrations of the peritoneal wash were maintained at approximately 7 mg. per 100 ml. except for the noted fluctuations during the first 24 hours. Despite the continual administration of a calcium free wash, the calcium concentrations after peritoneal equilibration were constant in the parathyroidectomized animals, but reduced to approximately 3.5 mg. per ml. per 100 ml. A drop in phosphate and citric acid concentrations similar to that for calcium occurred after parathyroidectomy. These changes could be noted by the second hour after removal of the parathyroids. As little as 6 I.U. of parathyroid extract administered over a four hour period was able to raise calcium, phosphate and citric acid concentrations to the control level.

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THE UPTAKE OF I^{131}-LABELED THYROXINE AND TRIIODOTHYRONINE BY THE NEUROHYPOPHYSIS[1]

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THE localization of I^{131} in the hypophysis of rabbits injected with I^{131}-labeled thyroxine was first reported by Joliot, Courrier, Horeau and Sue (1). This observation, which suggested a possible mechanism by which the level of circulating thyroxine controls the secretion of thyrotropin by the anterior pituitary, was confirmed by other workers (2–9) Surprisingly, however, it was found by all these investigators that the site of greatest concentration of I^{131} was not the anterior pituitary, but rather the neurohypophysis. For example, Jensen and Clark (3) examined the I^{131}-activity in different parts of the brain of normal and thyroidectomized rabbits injected with radiothyroxine, and, using both counting and radioautographic technqiues, found that the I^{131} localized in the neural lobe of the pituitary and in the median eminence of the tuber cinereum, but not in the anterior pituitary.

In contrast with other investigators, Jentzer (10) reported definite localization of I^{131} in the posterior lobe of the rabbit pituitary at intervals as early as seven hours after the injection of iodide-I^{131}. He reported that such accumulation was abolished by previous thyroidectomy, and concluded that iodide must first be converted to thyroxine before it is localized in the posterior pituitary.

The present communication is concerned primarily with the following aspects of the uptake of I^{131} by the hypophysis: (1) the uptake of I^{131} by the hypophysis of various species other than the rabbit, following injection of I^{131}-labeled thyroxine; (2) the chemical nature of the I^{131} localized in the posterior pituitary of rabbits injected with I^{131}-thyroxine, I^{131}-triiodothyronine, or I^{131}-iodide; (3) the effect of procedures calculated to change pituitary activity on the localization of I^{131} in this tissue.

METHODS

Preparation of I^{131}-labeled L-thyroxine

This has been described in detail elsewhere (11). The preparations used here contained 5 to 10% of their I^{131} in the form of triiodothyronine, as determined by filter paper

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chromatography. For injection into animals, the radiothyroxine was dissolved in a minimum of .02N NaOH and diluted with 5 or 10 volumes of isotonic $NaHCO_3$ solution. The specific activity was 3–5 μc. per μg. ($3–5\times10^4$ cts./sec./μg. on our scintillation counter).

Preparation of I^{131}-labeled L-triiodothyronine

Triiodothyronine exchanged very readily with I^{131} under the same conditions used for radiothyroxine preparation. The radioactive triiodothyronine was crystallized from the exchange mixture and separated, by filter paper chromatography, from a small percentage of I^{131}-thyroxine also formed during the procedure. The purified compound was eluted from the paper with 0.02N NaOH, and reprecipitated by acidification. It was dissolved in a minimum of 0.02N NaOH, and diluted with isotonic $NaHCO_3$ for injection. The specific activity was approximately 2 μc. per μg.

Preparation of I^{131}-labeled monoiodo-DL-thyronine

For purposes of comparison with I^{151}-thyroxine and I^{131}-triiodothyronine, one other I^{131}-derivative of thyronine was prepared which, from its method of preparation, was assumed to be 3′-mono-iodothyronine. This compound was made by treating 1 mg. of DL-thyronine (3.7 μmols., Hoffman-LaRoche), dissolved in 1 ml. of 7N NH_4OH, with 1 mg. (3.9 μmols.) of I_2^{131}. The quantity of I_2 added was just slightly in excess of the theoretical amount required to produce a monoiodo derivative. Chromatographic analysis of the reaction mixture revealed one major organic-I^{131} component which was eluted from the chromatogram with 0.05N NaOH and diluted with isotonic $NaHCO_3$ for injection. Its specific activity was 1.5 μc. per μg. of iodine. The product was not prepared in sufficient amounts to allow positive identification by the usual chemical procedures. However, on paper chromatograms, it assumed a position in the neighborhood occupied by other known iodinated thyronines, but distinct from that of thyroxine, triiodothyronine, or 3, 5-diiodothyronine. Other known iodothyronines were not available for comparison. From the fact that the iodination procedure was calculated to produce only a monoiodo derivative, and since iodination of the 3 or 5 position alone seems most unlikely, we assume that the thyronine derivative prepared here was 3′-monoiodothyronine.

Injection of animals and removal of tissues

All injections were made intravenously—in the rabbits and guinea pigs via the ear veins, in the dogs and cats via the saphenous vein, and in the rat via the tail vein. At the time of sacrifice (usually 4–5 hours), the animals were injected intravenously with nembutal, blood samples were taken from the inferior vena cava, and the heads were perfused with isotonic saline via the carotid artery. During the removal of the brain, the midpoint of the pituitary stalk was carefully cut with a pair of fine scissors. The anterior and posterior lobes of the pituitary gland and the median eminence of the tuber cinereum were separately dissected and rapidly weighed on an analytical balance. Samples from other regions of the brain (and in some experiments, other tissues) were also dissected and accurately weighed on a torsion balance. Immediately after they were weighed, the tissues were placed in small vials, for assay of radioactivity in a well-type scintillation counter with a sensitivity of 1×10^4 cts./sec. per μc. of I^{131}. The use of such a counter obviated any correction for mass absorption.

Preparation of tissue for chromatography

After they had been assayed for I^{131}-activity, the posterior pituitaries from several rabbits were pooled and homogenized with 100 μl. of alkaline saline (.02% NaOH). Five μl. of thiouracil solution (10 mg./ml.) were added, and the homogenate was then de-

livered on filter paper strips as described in a previous communication (12). Forty μl. were delivered in two 20-μl. portions. The chromatographing solvent was either collidine-water-ammonia or butanol-ethanol-2N NH_4OH (5:1:2). In some experiments, thyroxine or triiodothyronine markers were added, and the chromatograms were sprayed with diazotized sulfanilic acid.

Procedures calculated to result in different states of pituitary activity

The effect of different states of pituitary activity on the uptake of I^{131} by the pituitary was investigated. The following experimental procedures were used:

1) *Stalk section*—The pituitary stalk was cut in rabbits by the fronto-temporal approach of Westman and Jacobsohn (13), and a large waxed paper plate was inserted between the cut ends of the stalk. This procedure results in marked atrophy and cessation of function of the neurohypophysis and a decreased acactivity of the adenohypophysis (14). Radioactive thyroxine was injected 5–8 days after the stalk had been sectioned.
2) *Thyroidectomy*—Rats and rabbits were surgically thyroidectomized and injected with I^{131}-thyroxine 24 and 36 days later, respectively.
3) *Massive doses of alcohol*—This has been shown to reduce secretion of antidiuretic hormone by the posterior pituitary of the rabbit (15). The dose used was the same as that used by Cross and van Dyke (16)—5 ml. per kg. body weight, administered orally as a 12.5% v/v solution in water. The rabbits received an injection of radiothyroxine 30 minutes after administration of the alcohol.

RESULTS

Comparison of different species

Table 1 shows the uptake of I^{131} by the anterior pituitary, posterior pituitary and median eminence of the tuber cinereum of several species of animals injected with I^{131}-labeled thyroxine. At the interval tested—4–5 hours—only the rabbit and the dog showed a marked concentration of I^{131} by the posterior pituitary. In these animals, this tissue attained a concentration of I^{131} 21 to 55 times as great as that of the cerebral cortex, and 2 to 3 times as great as that in plasma. The other parts of the hypophysis did not show any unusual uptake of I^{131}. In the cat, guinea pig and rat, even the posterior pituitary did not concentrate I^{131} above the plasma level.

TABLE 1. COMPARISON OF HYPOPHYSEAL UPTAKE OF I^{131}-THYROXINE IN VARIOUS ANIMAL SPECIES

Species	No. of animals	Microgram thyroxine injected	I^{131} concentration relative to cerebral cortex			I^{131} concentration relative to plasma		
			In posterior pituitary	In anterior pituitary	In median eminence	In posterior pituitary	In anterior pituitary	In median eminence
Dog	3	29	34.3 (21–51)*	6.9 (4.8–9.0)	13.9 (6.0–20)	2.4 (2.0–2.7)	0.5 (0.5–0.6)	1.0 (0.6–1.3)
Rabbit	3	36	34.4 (22–55)	4.4 (3.2–5.6)	4.8 (3.4–6.3)	2.8 (2.0–3.4)	0.3 (0.3–0.4)	0.4 (0.3–0.4)
Cat	3	15	3.3 (2.8–3.9)	3.7 (3.5–3.9)	4.7 (4.5–5.0)	0.3 (0.3–0.4)	0.3 (0.3–0.4)	0.4 (0.3–0.5)
Guinea Pig	3	7	1.8 (1.5–2.2)	1.8 (1.6–2.4)	1.5 (1.1–1.9)	0.3 (0.2–0.4)	0.3 (0.2–0.3)	0.2 (0.2–0.4)
Rat	3	4	4.6 (3.5–6.2)	3.9 (3.3–4.4)	10.2 (6.6–14)	0.2 (0.2–0.3)	0.2 (0.2–0.2)	0.4 (0.3–0.5)

* Mean value and range.
The animals were sacrificed 4–5 hours after intravenous injection of the radioactive thyroxine.

TABLE 2. RADIOACTIVITY IN THE HYPOPHYSIS OF RABBITS AT VARIOUS TIMES AFTER INTRAVENOUS INJECTION OF I^{131}-THYROXINE

Hours after injection	I^{131} Concentration relative to cerebral cortex			I^{131} Concentration relative to plasma		
	In posterior pituitary	In anterior pituitary	In median eminence	In posterior pituitary	In anterior pituitary	In median eminence
2	47.4	7.3	6.5	2.8	0.5	0.4
4	55.4	4.3	7.0	4.8	0.4	0.6
8	18.3	3.7	5.0	2.1	0.4	0.6
17	23.4	2.5	5.4	3.7	0.4	0.9
30	33.3	4.0	5.3	5.5	0.7	0.8

Values for each interval are the averages obtained from two animals, each injected with 43 μg. L-thyroxine labeled with 118 μc. of I^{131}.

Time-study of the uptake of I^{131}-thyroxine by the posterior pituitary of the rabbit

Table 2 shows the relative concentrations of I^{131} in various portions of the hypophysis at different intervals after the injection of I^{131}-thyroxine. The posterior pituitary was the most active area at all the intervals tested. The peak values occurred at approximately 4 hours, although a second peak was suggested at a later interval. On the basis of these findings, all subsequent experiments were terminated at the 4–5 hour interval.

Uptake of I^{131}-thyroxine in various tissues of the rabbit

Table 3 shows average values for the relative concentration or I^{131} in a variety of tissues taken from rabbits 4 hours after injection of I^{131}-thyroxine. Of all the tissues shown, only the posterior pituitary concentrated I^{131} above the level in the plasma. The anterior pituitary and the

TABLE 3. COMPARISON OF RADIOACTIVITY IN VARIOUS TISSUES OF THE RABBIT 4 HOURS AFTER INJECTION OF I^{131}-THYROXINE

Tissue	Number of animals represented	Average weight of tissue taken	I^{131} concentration relative to cerebral cortex	I^{131} concentration relative to plasma
		mg.		
Posterior pituitary	15	4.4 ±0.9*	45 ±13	2.9 ±1.3
Anterior pituitary	15	26 ±4.6	5.0 ± 1	0.30 ±0.08
Median eminence	15	9.0 ±4.0	8.5 ± 4.2	0.48 ±0.17
Cerebral cortex	15	141	1	0.06 ±0.02
Thalamus	7	32	1	0.07
Posterior hypothalamus	4	24	1	0.06
Anterior hypothalamus	4	28	1.4	0.07
Caudate nucleus	7	30	1.7	0.11
Basis pedunculi	3	23	0.9	0.07
Pineal	6	9.4	5.5	0.28
Masseter	4	213	2.3	0.15

* Mean ±S.D. (S.D. = $\sqrt{\frac{\Sigma d^2}{n}}$).

median eminence concentrated I^{131} to a greater extent than did other areas of the brain such as cerebral cortex and hypothalamus. The pineal gland also showed a high concentration of I^{131} relative to cerebral cortex (see Reiss *et al.*, 1949).

Uptake of I^{131} by the hypophysis of rabbits injected with I^{131}-triiodothyronine, I^{131}-monoiodothyronine or I^{131}-iodide

Table 4 shows results of experiments comparing I^{131}-thyroxine with various other I^{131}-containing compounds.

(a) *I^{131}-triiodothyronine.*—The results obtained with this compound were similar to those obtained with thyroxine, in that the posterior pituitary again showed much the highest concentration of I^{131}. The concentration ratio for this tissue (compared with plasma) was 40–50, much higher than in the case of I^{131}-thyroxine. Similar results were obtained by Milofsky (personal communication, 1954). However, the concentration of I^{131} relative to plasma was much greater in all tissues following injection of I^{131}-triiodothyronine than after injection of I^{131}-thyroxine. Even the cerebral cortex attained a higher concentration of I^{131} than that of plasma following injection of this compound. The rapid disappearance of injected I^{131}-triiodothyronine from plasma and its rapid penetration into tissues (as contrasted with I^{131}-thyroxine) have been noted by others (17–20). As shown in the last two columns of Table 4, the total uptake of the injected dose by the posterior pituitary was only slightly greater in the case of triiodothyronine than in the case of thyroxine.

(b) *I^{131}-monoiodothyronine.*—The preparation of this compound was described by Roche and Michel (21), but no details are available concerning its physiological activity. It is likely, however, that the compound possesses little or no thyromimetic activity, and it seemed of interest to measure the concentration of I^{131} in the hypophysis after injection of I^{131}-monoiodothyronine. The data of Table 4 indicate that its metabolic fate is somewhat different from that of thyroxine or triiodothyronine. The concentration of I^{131} in the posterior pituitary was only slightly higher than that of the other portions of the hypophysis following injection of I^{131}-monoiodothyronine, and the percentage uptake of the injected dose was far less than it was after thyroxine or triiodothyronine injection.

(c) *I^{131}-iodide.*—A surprisingly high concentration of I^{131} was present in the posterior pituitaries of some rabbits 4–5 hours after injection of iodide I^{131}. The results were quite variable, a finding which may have been related to variations in the effectiveness of the saline perfusion. This would not explain, however, the finding that in 2 rabbits the concentration of I^{131} in the posterior pituitary reached values slightly higher than those of the plasma. The administration of a large dose of thiouracil did not affect the uptake of iodide-I^{131} by the neurohypophysis (Table 4). There appears to be little reason to suspect therefore, that formation of organic I^{131} was

TABLE 4. RADIOACTIVITY IN THE HYPOPHYSIS OF RABBITS AFTER INJECTION OF VARIOUS I¹³¹-CONTAINING COMPOUNDS

Compound	μg. Injected	Hours after injection	No. of animals	I¹³¹ concentration relative to cerebral cortex			I¹³¹ concentration relative to plasma			Uptake of injected compound by posterior pituitary	
				In posterior pituitary	In anterior pituitary	In median eminence	In posterior pituitary	In anterior pituitary	In median eminence	Per cent of injected I¹³¹	μ mols of injected compound
										per mg. tissue ×10⁴	per mg. tissue ×10⁷
L-Thyroxine	18	4–5	4	53 (44–61)*	4.7 (4.2–5.3)	11.3 (6.7–18.4)	2.8 (1.3–4.3)	0.2 (0.1–0.3)	0.5 (0.4–0.6)	8.0	1.8
L-Triiodothyronine	10	2–5	6	35.5 (28.4–45.3)	3.6 (2.5–4.7)	2.5 (2.0–2.8)	45.8 (38.1–56.4)	4.5 (3.6–6.2)	3.1 (2.6–3.7)	15	2.3
D,L-Monoiodothyronine	29	2–5	4	7.8 (3.3–16.2)	3.1 (2.5–3.5)	3.5 (2.7–5.0)	0.8 (0.4–1.6)	0.3 (0.3–0.4)	0.4 (0.3–0.5)	0.2	0.2
Iodide	carrier free	4–5	4	24 (13–14)	10 (8.5–11.4)	3.4 (2.1–4.1)	0.7 (0.4–1.2)	0.3 (0.27–0.32)	0.1 (0.06–0.12)	0.3	
Iodide† (+Thiouracil)	carrier free	4–5	2	28 (10–45)	10.5 (8.5–12.5)	3.3 (2.4–4.1)	0.7 (0.3–1.2)	0.3 (0.26–0.33)	0.1 (0.07–0.11)	0.6	

* Mean value and range.
† These rabbits were injected intraperitoneally with 100 mg. of thiouracil 45 minutes before administration of the radioactive iodide.

responsible for the results obtained after iodide-I^{131} injection. Evidence from filter paper chromatography, as described in the next section, also supports this conclusion.

Filter paper chromatographic analysis of the I^{131} taken up by the posterior pituitary

The nature of the I^{131} in the posterior pituitary of rabbits 4 hours after injection of I^{131}-thyroxine is revealed by the radioautographs of filter paper chromatograms shown in Figures 1B and 1C. Figure 1A shows the injected dose for comparison. It is clear that thyroxine itself, and not a transformation product, concentrates in the posterior pituitary of rabbits injected with I^{131}-thyroxine. Similarly, after injection of I^{131}-triiodothyronine, the I^{131} which accumulates in the posterior pituitary is identical with the compound injected (Fig. 2). Both thyroxine and triiodothyronine, therefore are concentrated unchanged by the posterior pituitary of rabbits.

In one experiment the nature of the I^{131} in the posterior pituitary 4 hours after administration of iodide-I^{131} was examined by filter paper chromatography. The I^{131} under these conditions was entirely inorganic. Examination of the plasma I^{131} at this interval by filter paper chromatography indicated no detectable organic I^{131} in the circulation.

Effect of experimental procedures, calculated to result in different states of pituitary activity, on the uptake of I^{131}-thyroxine by the hypophysis

1) *Pituitary stalk section.*—This resulted in a marked decrease in the weight of the posterior pituitary, presumably accompanied by cessation of function of this gland. Surprisingly, however, the operation did not interfere with the ability of the atrophic neural lobe to concentrate I^{131}-thyroxine (Table 5). Normal concentration ratios were observed in 4 different rabbits, operated upon 5–8 days previously, and in which a waxed paper plate had been inserted to intervene between the hypothalamus and pituitary gland.

   There was only little, if any, effect of stalk section on the weight of the anterior pituitary, and the uptake of I^{131}-thyroxine by this tissue was not different from that in normal rabbits. In the case of the median eminence also, the weight of the tissue was not changed by stalk section, and the uptake of I^{131} was in the normal range.
2) *Administration of alcohol.*—Inhibition of posterior pituitary function by administration of a large dose of ethyl alcohol had no observable effect on the ability of the neural lobe to concentrate I^{131}-thyroxine (Table 5).
3) *Thyroidectomy.*—Because thyroidectomy is known to produce basophilism in the anterior pituitary and a probable increase in the rate

of discharge of thyrotropin, it seemed of interest to compare the uptakes of I^{131} in the hypophyses of normal and thyroidectomized animals injected with I^{131}-thyroxine. The results are shown in Table 6. Thyroidectomy had little or no effect on the uptake of I^{131} by the anterior pituitary, but it seemed to increase the uptake of I^{131} by the

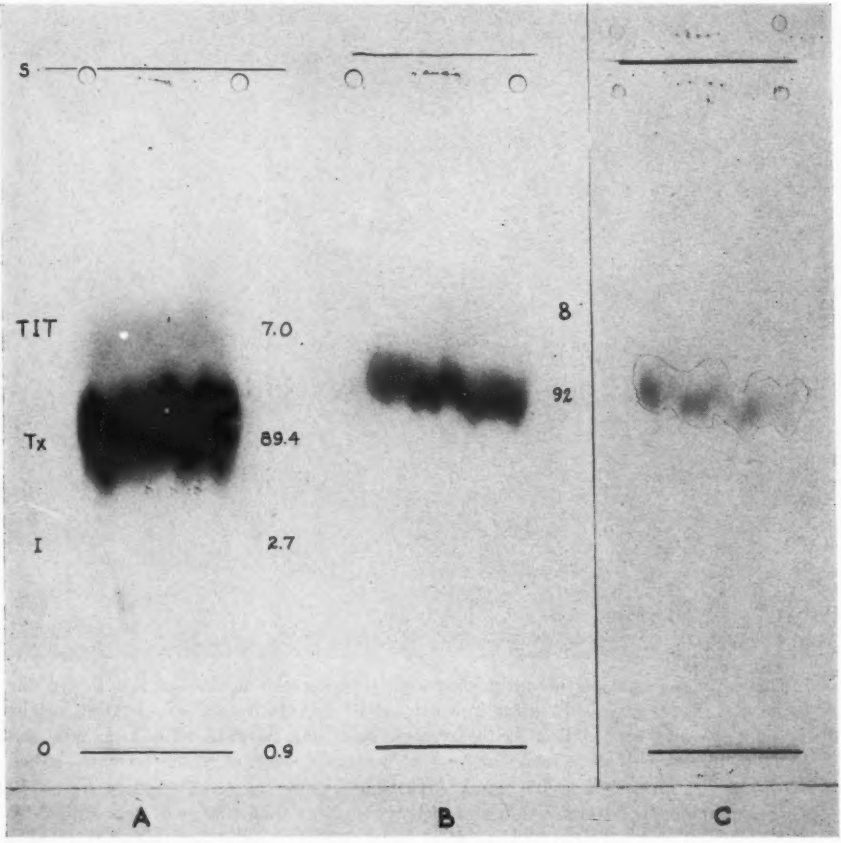

FIG. 1. Radioautographs of chromatograms showing the nature of the I^{131} in the posterior pituitaries of rabbits after injection of I^{131}-thyroxine. A) 20 μl. of the I^{131}-thyroxine preparation (diluted 1:5 with rabbit plasma). B) 40 μl. of homogenate prepared from the pooled posterior pituitaries of 6 rabbits, each of which received 18 μg. of I^{131}-thyroxine 4 hours before. C) Original paper chromatogram corresponding to Fig. 1B, showing the position of added thyroxine carrier as determined by spraying with diazotized sulfanilic acid. Numbers indicate the percentage of the I^{131} on the chromatogram in the various components. O, origin; I, iodide; Tx, thyroxine, TIT, triiodothyronine; S, solvent front. Chromatographing solvent: butanol-ethanol-2N ammonium hydroxide, 5:1:2.

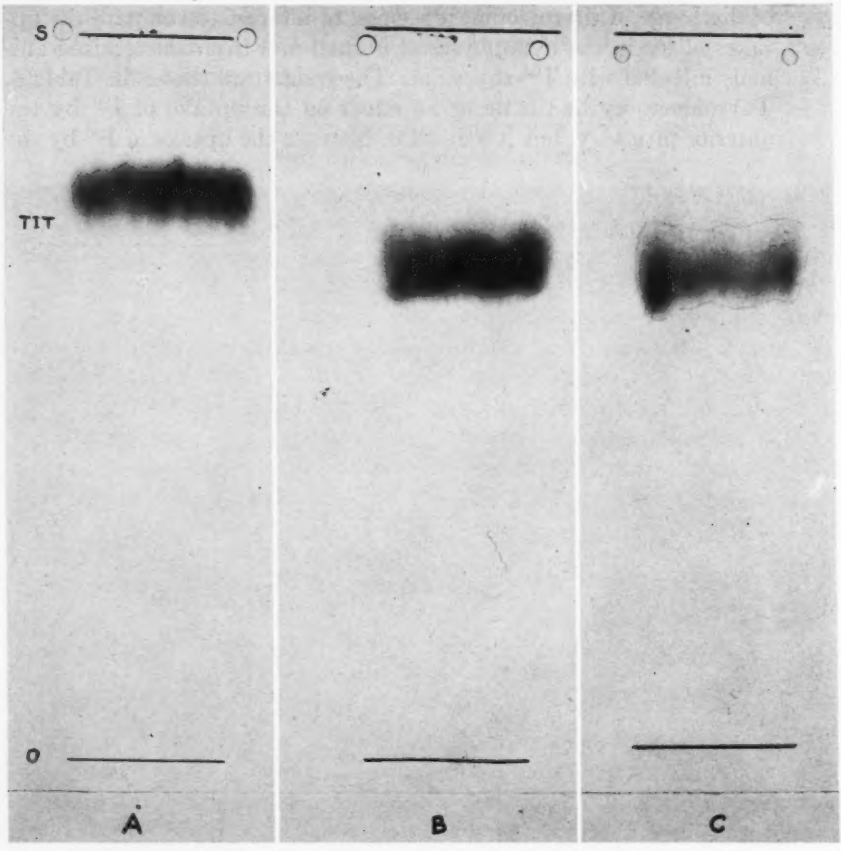

FIG. 2. Radioautographs of chromatograms showing the nature of the I^{131} in the posterior pituitaries of rabbits after injection of I^{131}-triiodothyronine. A) 20 μl. of the I^{131}-triiodothyronine preparation (in bicarbonate solution). B) 40 μl. of homogenate prepared from the pooled posterior pituitaries of 6 rabbits, each of which received 10 μg. of I^{131}-triiodothyronine 2–5 hours before. C) Original paper chromatogram corresponding to Fig. 2B, showing the position of added triiodothyronine carrier as determined by spraying with diazotized sulfanilic acid. O, origin; TIT, triiodothyronine; S, solvent front. Chromatographing solvent: butanol-ethanol-2N ammonium hydroxide 5:1:2.

neural lobe, especially in rabbits. There was no significant effect of thyroidectomy on the weight of the posterior lobes in these experiments, although there was definite enlargement of the adenohypophysis.

DISCUSSION

In the present investigation, we have confirmed the observation of previous workers that the I^{131} which is concentrated in the hypophysis of rabbits following the injection of I^{131}-thyroxine is localized primarily in the

TABLE 5. EFFECT OF PITUITARY-STALK SECTION OR ALCOHOL TREATMENT ON THE UPTAKE OF I^{131} BY THE HYPOPHYSIS OF RABBITS INJECTED WITH I^{131}-THYROXINE

Treatment of rabbit	Wt. of rabbit	Weight of tissue			I^{131} concentration relative to cerebral cortex			I^{131} concentration relative to plasma		
		Posterior pituitary	Anterior pituitary	Median eminence	Posterior pituitary	Anterior pituitary	Median eminence	Posterior pituitary	Anterior pituitary	Median eminence
	kg.	mg.	mg.	mg.						
Normal	2.2	4.7	31.0	6.1	42	5.8	11.6	2.4	0.33	0.67
Normal	2.3	5.9	29.2	3.9	61	7.4	15.2	3.3	0.40	0.81
Sham-operated	2.0	4.0	24.4	5.9	76	7.0	14.5	3.6	0.33	0.69
Sham-operated	2.5	3.9	16.0	4.0	43	7.6	19.3	1.9	0.34	0.86
Stalk-sectioned 5 days before receiving I^{131}-thyroxine	2.1	3.0	16.8	4.6	85	12	6.9	2.7	0.38	0.22
Stalk-sectioned 5 days before receiving I^{131}-thyroxine	2.7	2.0	18.4	6.7	36	8.9	10.4	1.9	0.47	0.54
Stalk-sectioned 8 days before receiving I^{131}-thyroxine	2.1	1.8	23.0	5.8	47	7.5	4.2	2.7	0.43	0.24
Stalk-sectioned 6 days before receiving I^{131}-thyroxine	2.6	2.9	22.4	4.8	64	7.0	11.6	3.0	0.33	0.54
Alcohol (5 gm. per kg.) administered orally 30 min. before injection of I^{131}-thyroxine	2.2	4.9	31.0	4.9	56	4.7	7.8	2.1	0.17	0.29
Alcohol (5 gm. per kg.) administered orally 30 min. before injection of I^{131}-thyroxine	2.4	4.8	23.9	3.9	55	4.6	10.6	2.1	0.18	0.40

TABLE 6. EFFECT OF THYROIDECTOMY ON THE UPTAKE OF I^{131}-THYROXINE BY THE HYPOPHYSIS OF RABBITS AND RATS

Animals	No. of animals	μg. thyroxine injected	I^{131} concentration relative to cerebral cortex			I^{131} concentration relative to plasma		
			In posterior pituitary	In anterior pituitary	In median eminence	In posterior pituitary	In anterior pituitary	In median eminence
Normal rabbits	4	18	53.0 (44–61)*	4.7 (4.2–5.3)	11.3 (6.7–18.4)	2.8 (1.3–4.3)	0.2 (0.1–0.3)	0.5 (0.4–0.6)
Thyroidectomized Rabbits	4	18	122 (88–158)	6.3 (4.4–10)	13.5 (10.1–16.6)	6.1 (2.6–13.1)	0.3 (0.2–0.4)	0.7 (0.3–1.4)
Normal Rats	3	5	4.6 (3.5–6.2)	3.9 (3.3–4.4)	10.3 (6.6–14)	0.2 (0.2–0.3)	0.2 (0.2–0.2)	0.4 (0.3–0.5)
Thyroidectomized Rats	3	5	7.4 (7.1–8.0)	4.0 (3.7–4.3)	4.7 (4.0–5.5)	0.4 (0.3–0.4)	0.2 (0.1–0.2)	0.2 (0.2–0.3)

* Mean value and range.

Thyroidectomized rabbits were taken 36 days, and thyroidectomized rats 24 days after removal of thyroid glands. All animals were sacrificed 4 to 5 hours after injection of radiothyroxine.

posterior pituitary. Under the conditions of our experiments, the concentration of I^{131} in the posterior pituitary attained values 20 to 60 times as great as that of the cerebral cortex or hypothalamus, and 2 to 4 times as great as that of plasma. The concentration of I^{131} in the anterior pituitary, on the other hand, was only 5–7 times as great as that in cerebral cortex, and was usually less than 0.5 the value in plasma. The median eminence of the tuber cinereum, which is part of the neurohypophysis (22), generally contained I^{131} in a concentration only slightly higher than that in the anterior pituitary, and always considerably lower than in the posterior pituitary. It must be remembered, however, that the median eminence, as measured here, was always dissected along with more or less extraneous tissue, and the I^{131} concentration obtained for this tissue was, therefore, probably too low.

Courrier (23) briefly reported that the radioactivity which accumulates in the posterior pituitary following injection of I^{131}-thyroxine is in the form of the administered compound. In the present investigation the nature of the I^{131} taken up by the posterior pituitary following injection of I^{131}-thyroxine or I^{131}-triiodothyronine was determined by filter paper chromatography. With each of these compounds, the I^{131} taken up by the tissue was chemically identical with the injected material. Both of these compounds, therefore, are concentrated unchanged by the posterior pituitary. Similar findings have recently been reported by Gross *et al.* (9).

An interesting difference between I^{131}-thyroxine and I^{131}-triiodothyronine appeared in the rates at which these compounds penetrated into central nervous tissue. I^{131}-thyroxine, after intravenous injection into rabbits, penetrated into cerebral cortex very slowly, attaining a concentration in this tissue approximately 5 per cent of that in plasma 4 hours after injection. I^{131}-triiodothyronine, on the other hand, rapidly entered the cells of the cerebral cortex, as indicated by a concentration ratio of well over one in 4 hours. Nervous tissue is no exception, therefore, to the well established finding that I^{131}-triiodothyronine rapidly enters most tissues of the body. The finding that I^{131}-triiodothyronine in posterior pituitary was present at a concentration 40–50 times as great as that in plasma 4 hours after injection (corresponding ratio for I^{131}-thyroxine, 2–4) is largely explained by the fact that the plasma level of I^{131}-triiodothyronine drops so rapidly. The actual percentage uptake of I^{131}-triiodothyronine in posterior pituitary tissue was only slightly greater than that of I^{131}-thyroxine (last column, Table 4).

The rabbit and the dog in the present series of experiments differed from the cat, rat and guinea pig in the ability of their posterior hypophysial tissue to concentrate thyroid hormone. Courrier (23) also reported marked species differences. Out of six species which he tested, only the monkey and the rabbit showed a higher concentration of I^{131} in the posterior pituitary than in the blood.

When it was first reported that I^{131} is concentrated in the hypophysis of rabbits injected with I^{131}-thyroxine, this observation appeared to have some relation to the control of thyrotropin secretion by the anterior pituitary. After it became clear that it is the posterior pituitary, and not the anterior pituitary, which is responsible for this concentration, some workers adopted the view that the neurohypophysis might be an essential part of the pituitary-thyroid axis, and that it might play a regulatory role in the production of thyrotropic hormone (3). This hypothesis is not easily reconciled with some of the findings of the present investigation. Foremost among these is the observation that sectioning of the pituitary stalk, a procedure which greatly inhibits posterior pituitary function in the rabbit, had no effect on the ability of this tissue to concentrate I^{131}-thyroxine. Similarly, the administration of massive doses of ethyl alcohol, which blocks ADH production by the posterior pituitary, did not affect the uptake of I^{131}-thyroxine.

On the other hand, our observation of an increased uptake of I^{131}-thyroxine by the posterior pituitary of thyroidectomized rabbits (5) requires some explanation. This finding appears to support the view that the posterior pituitary may be involved in the regulation of TSH output by the anterior pituitary. However, the interpretation of these results is complicated by the fact that the degree of dilution of injected I^{131}-thyroxine with endogenous thyroxine was different in the normal and in the thyroidectomized animals. Further studies are required, therefore, to assess the significance of the increased uptake of I^{131}-thyroxine by the posterior pituitary of thyroidectomized rabbits.

The sites where I^{131}-thyroxine concentrates (the pituitary and pineal glands, as compared with the rest of the brain) are similar to those in which vital dyes accumulate (22, 24). It is unlikely that the uptake of the latter is in any way connected with anterior pituitary function. Similarly, the uptake of I^{131}-thyroxine by the posterior pituitary may be non-specific and unrelated to anterior pituitary production of TSH. It remains to be explained, however, why a compound so similar to thyroxine as monoiodothyronine is not concentrated to any appreciable extent by the posterior pituitary.

The lack of concentration of I^{131}-thyroxine by the tissues of the central nervous system proper is similar to the lack of concentration of tritiated hexoestrol observed by Glascock and Harris (unpublished observations). In this work, ovariectomized rabbits were injected with labeled hexoestrol until their sexual behaviour was oestrous in type. Even with this evidence that the oestrogen was acting on some neural mechanism, no concentration of radioactivity could be detected in any part of the brain. However, there are no *a priori* reasons for believing that hormones which affect central nervous or hypophysial activity necessarily concentrate at their site of action.

During the course of the present investigation, several papers appeared dealing with the possible role of neurohypophysial secretions in regulating ACTH output by the anterior pituitary (25, 26, 27). These results are still inconclusive, however, and further work is required to establish any physiological relationship between humoral factors from the posterior pituitary and ACTH production.

SUMMARY

1. Following the injection of I^{131}-labeled thyroxine into rabbits, dogs, cats, guinea pigs and rats, the uptake of I^{131} in excised portions of the hypophysis and others parts of the brain was measured with the aid of a scintillation counter. The uptake of I^{131} was similarly measured in rabbits that received I^{131}-triiodothyronine.

2. In rabbits and dogs that received I^{131}-thyroxine, the concentration of I^{131} in the posterior pituitary attained (in 4 hours) values that were 20–60 times as great as the concentration of I^{131} in the cerebral cortex, and 2–4 times as great as the concentration of I^{131} in plasma. The anterior pituitary and the median eminence of the tuber cinereum did not concentrate I^{131} above plasma levels. In the cat, guinea pig and rat, no concentration of I^{131} above plasma levels occurred in any part of the hypophysis.

3. I^{131}-triiodothyronine penetrated into all parts of the brain much more rapidly than did I^{131}-thyroxine. After injection of the former into rabbits, the I^{131} concentration in the posterior pituitary attained values 40–50 times as high as the plasma level. The total uptake of I^{131} by this tissue, however, was only slightly greater than after injection of I^{131}-thyroxine.

4. The nature of the I^{131} concentrated by the posterior pituitary of the rabbit was examined by filter paper chromatography. It was observed that both I^{131}-thyroxine and I^{131}-triiodothyronine are concentrated unchanged by the neurohypophysis.

5. Section of the pituitary stalk in rabbits resulted in atrophy of the posterior pituitary, but had no effect on the ability of this tissue to concentrate I^{131} after injection of I^{131}-thyroxine. Inhibition of posterior pituitary function with massive doses of alcohol also did not affect the ability of the posterior pituitary to concentrate I^{131}-thyroxine. On the other hand, surgical thyroidectomy seemed to increase the ability of the posterior lobe to concentrate I^{131}-thyroxine.

6. The significance of these findings is discussed in relation to a possible regulatory action of the neurohypophysis on anterior pituitary activity.

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INGUINAL HERNIAS IN FEMALE MICE TREATED WITH ANDROGENS OR BEARING GRAFTS OF TESTES[1]

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SCROTAL hernias appeared in male mice that received estrogen (1, 2' 3). Inguinal hernias have been observed occasionally in irradiated, castrated or intact female mice of certain stocks bearing subcutaneous grafts of testes from immature donors. Inguinal hernias also developed in mice that were treated with estrogens while bearing subcutaneous transplants of testicular interstitial cell tumors. The latter observations have been made sporadically over a period of ten or more years and incidental to other experiments. The time of first appearance of the hernias and the size of the hernias was not determined. Many small hernias probably were not noted because the mice were not specifically followed for hernias. Hernias of large size did appear frequently among female mice of some groups treated as indicated above and some examples are summarized in Tables 1 and 2.

Critical evaluation of the hormones produced by the testicular grafts or the tumor transplants were not done but androgenic hormones of some type were very probably produced as indicated by the interpubic ligament responses in female mice and the condition of the seminal vesicles and prostates of males bearing transplants of the interstitial cell tumors (4).

Some studies specifically directed toward the influences of testicular transplants and of androgenic hormone treatment on the development of inguinal hernias[3] in female mice are reported here.

MATERIALS AND METHODS

Hybrid female mice were used for these experiments. They were designated CB (BC strain$\times$C$_{57}$ strain) and the CC (C$_{57}$ strain$\times$CBA strain). Female mice of these stocks

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[2] U.S.P.H.S. Summer fellow 1955.

[3] The term "hernia" as used hereafter refers to any extension of the peritoneum and adjacent musculature and fascia caudal and ventral to the lateral borders of the rectus abdominis near and at its pubic origin. A slight weakening of the fascial and muscular tissues may occur normally but will not permit a ventro-caudal extension of more than 1 mm. This probably should not be called a hernia but the term as used here includes even this extension. Current investigation of the early morphogenesis of hernia formation in the mouse indicates the necessity of this definition.

TABLE 1. INCIDENCE OF INGUINAL HERNIAS IN IRRADIATED FEMALE MICE BEARING TRANSPLANTED TESTIS OF IMMATURE DONOR FOR PERIODS LONGER THAN SIX MONTHS*

Stock	Animals treated number	Hernias in treated mice	
		Number	Per cent
CC_1 (C_{57}×CBA)	24	13	54.2
CC_2 (CBA×C_{57})	5	3	60.0
CB_1 (BC×C_{57})	15	4	26.7
CB_2 (C_{57}×BC)	25	7	28.0
A_{71} (A×C_{57})	8	2	25.0
A_{72} (C_{57}×A)	9	0	0.0
BC	12	3	25.0

* These data were obtained from observations on experimental groups not specifically followed for incidence of hernia formation.

showed a high incidence of hernias when bearing immature testes transplanted subcutaneously (Table 1).

Untreated female mice were dissected to develop methods for objective evaluation of progressive stages of inguinal herniation. After the animals were killed using illuminating gas, 10 ml. of 10% formalin were injected into the peritoneal cavity. Since both treated and untreated mice were of approximately the same sex and age, a uniform intra-abdominal pressure should have been achieved.

The skin and superficial layers of abdominal fascia, with their variable amounts of adipose tissue, were removed from the lower abdomen and perineum. This permitted clear visualization of the lower abdominal musculature. Mice with small bilateral hernias showed no gross abnormalities until after the adipose tissue was removed medial to the inguinal canals and ventral to the rectus abdominis.

TABLE 2.* INCIDENCE OF INGUINAL HERNIAS IN FEMALE MICE BEARING TRANSPLANTED TESTICULAR INTERSTITIAL CELL TUMORS AND STILBESTROL CHOLESTEROL PELLETS† SUBCUTANEOUSLY

Stock	Animals treated, No.	Days between onset and end of treatment	Tumor donor‡	Treated mice with hernias, No.
AC_1 (A×C_3H)	25	> 90	54 AC_2 TACE II§	0
AC_1 (A×C_3H)	3	±120	82 AC_2 TACE II‖	0
AC_1 (A×C_3H)	5	±270	107 AC_2 TACE II¶	1
AC_2 (C_3H×A)	64	> 90	54 AC_2 TACE II	0
AC_2 (C_3H×A)	2	±120	82 AC_2 TACE II	0
AC_2 (C_3H×A)	21	± 16	82 AC_2 TACE II	0
AC_2 (C_3H×A)	11	± 30	82 AC_2 TACE II	6
AC_2 (C_3H×A)	24	±270	107 AC_2 TACE II	0

* The data were obtained from observations on experimental groups not specifically followed for incidence of hernia formation.

† One part stilbestrol to three parts cholesterol: average wt. =8 mg.

‡ The donor is the original mouse in which the tumor appeared. Some of the tumors have been carried through several transplant generations.

§ Original tumor appeared in mouse 54 AC_2 ♂ that had received 100 μg. tri-para-anisyl-chloroethylene (TACE) weekly dissolved in 0.05 cc. of sesame oil. Injections were continued for 80 weeks. TACE was generously supplied by the W. S. Merrill Company, Cincinnati, Ohio.

‖ Original tumor appeared in mouse 82 AC_2♂ that had received 100 μg. TACE weekly dissolved in 0.05 cc. of sesame oil. Injections were continued for 73 weeks.

¶ Original tumor appeared in mouse 107 AC_2 castrate ♂ bearing a testis from a 24 hour old A ♂ and a pellet of stilbestrol-cholesterol subcutaneously (Unpublished data of Dr. J. J. Trentin).

In experimental animals the distance was measured in millimeters from the caudal border of the pubic symphysis or interpubic ligament to the most caudal extension of the hernia. This afforded a basis for comparison of the effects of different androgenic agents in eliciting herniation in female mice. Figures 1 and 2 illustrate the type of variation encountered in experiments completed to date.

FIG. 1. Female mouse dissected as described. Note external inguinal ring in relation to pubic tubercles and lateral borders of the rectus abdominis. No hernia.

FIG. 2. Female mouse dissected to show early inguinal hernias. Note disappearance of pubic tubercles and origin of the rectus abdominis medial to bilateral inguinal hernias. Size of hernia—4 mm.

TABLE 3. INCIDENCE OF INGUINAL HERNIAS IN FEMALE MICE BEARING A TESTIS FROM AN IMMATURE DONOR TRANSPLANTED SUBCUTANEOUSLY IN THE RIGHT LATERAL MARGIN OF THE HYPOGASTRIC REGION

Stock	Age of mouse at time of transplant (days)	Time between transplantation and death of mouse (days)	Size of hernia (mm.)	
			Left	Right
CB_2 ($BC \times C_{57}$)	196	85	4	4
CB_2	70	29	2	2
CB_2	70	29	1	1
CB_2	70	43	0	0
CB_2	70	30	3	5
CB_2	70	30	2	3
CB_2*	196	87	1	1
CB_2*	104	92	6	6
CB_1* ($C_{57} \times BC$)	195	149	6	4

* Indicates castrated animal.

OBSERVATIONS

Hernias were found in mature intact and castrated female mice bearing an immature testis[4] transplanted subcutaneously into the right lateral margin of the hypogastric region[5] (Table 3). The hernias were first observed in the adult mice that were dissected approximately 30 days after the testis was implanted. Larger hernias appeared in the mice bearing the grafts for 80 to 150 days. No mice included in Table 3 showed hernias before they were dissected. One animal still living shows gross evidence of small bilateral hernias 255 days after the testis was implanted.

Intact and castrated mature and immature female mice had pellets of testosterone (average weights of the pellets in the adult and immature mice were 3.8 and 1.9 mgm. respectively) implanted subcutaneously into the right lateral margin of the hypogastric region (Table 4). Mice in this group that were killed approximately 30 days after onset of treatment showed hernias. Mice killed at longer intervals after transplantation had smaller hernias. From 75 to 90% of the testosterone pellets were absorbed during the first 30 days after implantation into mature mice.

Mature intact and castrated female mice received a testosterone pellet (avg. wt. = 3.8 mg.) subcutaneously in the right hypochondriac region (Table 5). Small inguinal hernias were observed in mice killed after the pellet had been in place approximately 100 days. In animals killed after the pellet had been in place 165 to 210 days very small hernias, or no hernias, were observed.

In nine untreated castrated and intact female mice from 225 to 389 days old, none had hernias larger than 1 mm. These mice were observed at ages

[4] Testes from 2 to 12-day old mice of the same stock were used.

[5] In castrated animals the testis or testosterone pellet was transplanted 7 days after ovariectomy.

TABLE 4. INCIDENCE OF INGUINAL HERNIAS IN FEMALE MICE BEARING A TESTOSTERONE PELLET (AVE. WT. 3.8 MG.) SUBCUTANEOUSLY IN THE RIGHT LATERAL MARGIN OF THE HYPOGASTRIC REGION

Stock	Age of mouse at time of pellet implantation (days)	Time between implantation and death of mouse (days)	Size of hernia (mm.)	
			Left	Right
CB_2 ($BC \times C_{57}$)	180	180	4	4
CB_1 ($C_{57} \times BC$)	179	165	2	2
CB_2	43	29	5	5
CB_2	43	29	5	5
CB_2	54	43	1	1
CB_2	43†	43	5	5
CB_2	88	210	1	1
CB_1	133	165	1	1
CB_1	15‡	15	6	6
CB_1	15‡	15	7	7
CB_2*	180	103	7	7
CB_2*	180	109	1	1

* Indicates castrated mouse.
† Supplementary pellet implanted at same location 30 days after original implantation.
‡ Avg. pellet wt. 1.9 mg.

comparable to those of the hormone-treated animals which acquired larger hernias. Among 20 untreated mice dissected while undertaking a study of the morphology of the inguinal region in female mice, no hernias exceeding 1 mm. were found.

DISCUSSION

Hernias developed in mice when the hormonal environment was manipulated in a number of ways, presumably by increasing the levels of androgen. The quantitative aspects of the hormonal levels were not determined adequately. Androgens of exogenous origin (injected testosterone and transplants of normal and tumorous testes) stimulated hernia development.

TABLE 5. INCIDENCE OF INGUINAL HERNIAS IN FEMALE MICE BEARING A SUBCUTANEOUS TESTOSTERONE PELLET IN THE RIGHT HYPOCHONDRIAC REGION

Stock	Age of mouse at time of pellet implanted (days)	Time between implantation and death of mouse (days)	Size of hernias (mm.)	
			Left	Right
CB_2 ($BC \times C_{57}$)	180	103	2	2
CB_2	180	210	0	0
CB_2	146	210	0	0
CB_2	136	185	1	1
CB_2	105	109	4	4
CB_2	133	210	0	0
CB_2*	123	103	3	3
CB_2*	180	103	1	1
CB_2*	171	165	1	1
CB_2*	136	210	1	1

* Indicates castrated animal.

Castrated female mice in which estrogen stimulation was assumed to be minimal, developed inguinal hernias when bearing testosterone pellets or testis transplants. Androgen alone seemed to elicit hernias in these animals. However, animals castrated when 104 to 106 days of age had been subjected to the influence of intrinsic estrogens for some time before they were castrated. Because estrogen modified the interpubic tissues and consequently the muscular attachments thereto previous to castration (3), a residual effect must be considered.

In mice bearing testosterone pellets subcutaneously, uniformly smaller hernias were observed in most mice dissected from 60 to 120 days after the pellet was implanted than in mice bearing the pellet for less than 60 days. The regression of the hernias is thought to result from the absence of continued androgen stimulation since the pellets were largely absorbed during the first month after transplantation (5).

Female mice bearing both a transplanted testicular interstitial cell tumor and an estrogen pellet subcutaneously in opposite axillae developed inguinal hernias (Table 2). Hernias appeared after approximately 30 days.

Although the pellets were always implanted unilaterally in the region of one inguinal canal the responses were bilateral and approximately equal on both sides. The hormone induced no accentuated response on the ipsilateral side; systemic levels above the threshold limits of the response must have been attained.

Whether or not androgen acts directly on the musculature and fascia of the inguinal canal is not known. Testosterone had a myotrophic effect on the perineal musculature (bulbocavernosus, ischiocavernosus and levator ani) of rats (7).

Estrogens elicit a dissolution of the medial aspect of the pubic bones and a proliferation of an interpubic ligament (3). These latter changes are associated with the appearance of scrotal hernias in estrogen-treated male mice.

Androgen may have effects on muscles other than the perineal musculature. The temporalis muscles of guinea pigs undergo marked hypertrophy after androgen treatment (6). Sex differences in the abdominal musculature of mice have not been described. At the present time the appearance of the hernia cannot be ascribed to specific muscular, fascial or skeletal changes. The possibility was considered that these may not be hernias. The inguinal canals of male mice remain open throughout life and the testes may be retracted into the abdomen. Pressure upon the abdomen, however, fails to displace the abdominal viscera into the scrotum. When hernias appear in estrogen-treated males the viscera are almost always displaced into the scrotum in the living animal, and are readily forced into the scrota of dead animals. Also, among the females, viscera are found in the larger hernias. For these reasons it is assumed that true hernias are formed rather than merely a development of scrota in adult females. The smaller hernias con-

tain only the paramesometrial fat, and may not be unlike scrota. The possibility of a two stage process, namely, scrotum formation and then hernia formation, is being considered.

SUMMARY

Hernias appeared in female mice under the following experimental conditions: (1) Intact and castrated irradiated mice bearing immature testis transplants in the right axillae for periods of more than six months. (2) Mature mice bearing transplanted testicular interstitial cell tumors and an estrogen pellet subcutaneously in opposite axillae for approximately 30 days or more.

Inguinal hernias visible after special dissection were observed in mature and immature, castrated and intact female mice treated as follows: (1) Mice bearing a testosterone pellet subcutaneously in the right hypogastric or hypochondriac region for approximately 35 days. (2) Mice bearing an immature testis transplanted in the right hypogastric region for periods of 30 to 150 days.

No hernias larger than 1 mm. were observed in intact and castrated untreated female mice studied grossly or after special dissection. Hernias in all stages of development tended to be bilateral.

The role of androgen in eliciting inguinal hernia development in female mice has been demonstrated and the probability of estrogen as an essential co-factor considered.

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ADRENAL AND HYPOPHYSEAL INFLUENCES UPON THE UPTAKE OF RADIOACTIVE GOLD (AU^{198}) BY THE RETICULO-ENDOTHELIAL SYSTEM[1]

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PREVIOUS studies have established a relation between the adrenal cortex and the reticulo-endothelial system. Thus, adrenal insufficiency results in an impaired phagocytic capacity of the macrophagic elements of the spleen toward colloidal thorium dioxide (1, 2, 3). In addition, this diminished activity is prevented by the administration of certain adrenal cortical extracts or cortisone (1, 4, 5).

In extending these studies, it was deemed advisable to employ radioactive colloidal gold since it can be used in amounts sufficiently low to preclude any toxic effects; and because of its radio-chemical characteristics, its specific accumulation in the phagocytic elements of the reticulo-endothelial organs can be quantitated readily in these small doses (6, 7, 8, 9, 10, 11, 12, 13, 14, 15). Since the entrance of such an agent into the macrophage occurs normally only through actual ingestion, its use also averts the factor of diffusion, a criticism against the employment of vital dyes for such experiments (16). Moreover, considerable excretion of such dyes occurs, whereas gold colloids are eliminated in slight traces if at all (11, 17, 18, 19). The uniformity of particle size of radio-gold represents another advantage over other colloidal agents frequently used for identification of phagocytic cells *in situ*, such as India ink (20, 21, 22), manganese dioxide (23, 24), and chromium phosphate (25, 26, 27).

The purpose of this study has been to amplify and extend the quantitative approach in the elucidation of the phagocytic capacity of the macrophagic tissues, as affected by various endocrine procedures. It will describe

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the effects obtained with radioactive colloidal gold and consider the uptake of these particles in several tissues containing macrophagic elements.

MATERIALS AND METHODS

Young adult male albino Wistar rats, with initial weights ranging between 135 to 160 grams, were used in these studies. They were maintained on a well-balanced diet with access to drinking water at all times, and were kept in rooms which were thermostatically controlled at 75° ± 1° F. Bilateral adrenalectomies were performed by the dorsal, and hypophysectomies by the parapharyngeal approach. All animals were checked at autopsy to determine completeness of the operations; any found to be incomplete were discarded and not included in the data. The drinking fluid of adrenalectomized rats consisted of 1% NaCl whereas the hypophysectomized rats had access to 5% glucose. In addition, the diets of hypophysectomized rats were fortified with bread, milk, fresh meat, beef suet, fresh oranges, and raw carrots.

Cortisone (Compound E of Kendall) and corticosterone (Compound B of Kendall) were used as saline suspensions; lipo-adrenal extract, desoxycorticosterone acetate (DCA) and epinephrine as oil suspensions, and hydroxycorticosterone (Compound F of Kendall) in both saline and oil. A mixture of DCA and hydroxycorticosterone was prepared by adding finely ground crystalline hydroxycorticosterone to an oil suspension of DCA. Growth hormone (GH; Raben-Westermeyer preparation) was triturated, suspended in distilled water, and adjusted to pH 3. Adrenocorticotrophic hormone (ACTH; 20 units/mg.) was triturated and suspended in sesame oil containing 2% aluminum monostearate.

A stabilized colloidal sol of Au^{198} (Aurcoloid-198) with a specific activity of 5 microcuries per milligram was diluted with distilled water to obtain the desired activity. Measurement of the radioactivity of the injected dose was accomplished by diluting an aliquot, identical in volume to that injected, to such a level that a 1 cc. sample could be counted with a moderately high counting rate. Two such standards were prepared with each group of experimental animals receiving Au^{198}. Corrections for radioactive decay were circumvented by the simultaneous counting of the standards.

Entire spleens, lungs, and thymus glands, halves of livers for duplicate samples, pooled mesenteric, iliac, axillary, inguinal, and cervical lymph nodes, pooled gastrocnemius and thigh muscles, and approximately 3 cc. of blood were placed into previously weighed screw cap glass vials for counting. The solid organs were cut into small pieces and spread evenly on the bottom of the vials. The gamma radiation of each sample, including the standards, was then measured in a well-type scintillation counter. Most samples were sufficiently active to permit counts of one or two minutes' duration, and weaker samples were counted until at least 1000 counts were recorded. All samples were counted in triplicate and were reported as counts per minute. Background radiation was determined prior to and after each series of samples analyzed and was subtracted from the experimental measurements. After the determination of radioactivity, the samples were dried in a hot-air oven at 95–100° C for 24 hours; this allowed the concentration of Au^{198} to be expressed as counts per minute per gram of dry weight. Moreover, for valid comparisons between different experiments, the results were expressed as per cent uptake of the injected dose.

The total uptake by the organs was also determined. Since the whole spleen, liver, lung, and thymus were removed for analysis, the per cent of the injected Au^{198} could be evaluated for each set of experimental data. Two per cent of the average body weight was used as an estimate of the weight of the entire lymph system (28, 29). The assumption was made that the uptake of the whole lymph system would be proportional to that of the lymph nodes which were studied experimentally. The gold content of approxi-

mately 3 cc. aliquots of blood was determined throughout the experiment. The total blood volume of the normal, adrenalectomized and hypophysectomized rat was assumed to be 5, 4.8, and 4.5 cc. per 100 grams of body weight respectively (30, 31, 32, 33, 34). The total muscle content of the rat was assumed to be equivalent to 50% of the body weight (35).

In Series 1, the effects of adrenalectomy were tested upon the uptake of radioactive colloidal gold at various time intervals after its injection. Eight groups of normal and eight groups of adrenalectomized animals, each consisting of seven rats, were injected intravenously, via the jugular vein under light anesthesia, with approximately three microcuries of Au^{198} in 0.3 cc. volume twenty-four hours after adrenalectomy, and were sacrificed afterwards along with controls at the following time intervals: 0–1 minute, 15 minutes, 30 minutes, 1 hour, 1½ hours, 2 hours, 6 hours and 24 hours.

Three cc. of blood obtained by cardiac puncture and the spleens, livers, lungs, thymus glands, lymph nodes and muscle tissue were assayed for radioactivity. Results from normal and adrenalectomized animals at 1½ hours were not statistically different from, and consequently were pooled with, Groups 2A and 2B (see below), respectively.

In Series 2, the effects of adrenalectomy and of replacement treatment with various adrenal hormones were examined upon the uptake of Au^{198} by spleens, livers, and lungs at one and one-half hours after Au^{198} administration. Groups A (30 rats) and B (30) received no treatment; Groups C (25), D (25), and E (15) received two subcutaneous doses daily of 1.5 mg., respectively, of cortisone, hydroxycorticosterone and corticosterone in 0.1 cc. physiological saline for two days; Group F (30) was injected with saline only. Groups G (25 rats), H (15), I (15), and J (15) received subcutaneous doses, respectively, of 0.5 cc. lipo-adrenal extract (Upjohn), 2.5 mg. DCA, 1 mg. DCA plus 3 mg. hydroxycorticosterone and 200 μg. of epinephrine in 0.5 cc. oil once daily for 2 days; group K (30 rats) received sesame and/or cottonseed oil only. Since there was no significant difference between animals receiving either vehicle, these were pooled. On the third day, animals in group A were sham-operated; all other animals were adrenalectomized. Animals in groups C, D, E, and F received 5 similar doses of hormone and saline respectively, and animals in groups G, H, I, and J received 3 similar doses of hormones and K of oil, over the subsequent twenty-four hours.

All animals were then injected intravenously with approximately 3 microcuries of Au^{198} in 0.3 cc. volume, and were sacrificed by exsanguination one and one-half hours later. The spleens, livers, and lungs were removed and analyzed for Au^{198} content.

In Series 3, the effects of hypophysectomy were tested on the uptake of radioactive colloidal gold at various time intervals after its injection. Five groups of normal and five groups of hypophysectomized animals, each consisting of seven rats, were injected intravenously with approximately 3 microcuries of Au^{198} in 0.3 cc. volume, and were sacrificed afterwards at the following time intervals: 0–1 minute, 15 minutes, 30 minutes, 1½ hours and 6 hours.

As in the previous series, animals were sacrificed by removal of blood via heart puncture; 3 cc. of this blood and the spleens, livers, lungs, thymus glands, lymph nodes, and muscle tissue were assayed for radioactivity. Results from normal and hypophysectomized animals at 1½ hours were not statistically different from, and consequently were pooled with groups 4A and 4B (see below), respectively.

In Series 4, the effects of hypophysectomy and of replacement treatment with adrenal and hypophyseal hormones were examined upon the uptake of Au^{198} by spleens, livers, and lungs. Eight groups of Wistar rats, each consisting of 15 animals, were used at 24 to 28 days after hypophysectomy. Group A consisted of normal intact animals. Groups B through H were hypophysectomized. Groups A and B received no treatment; groups C, D, E, F, G, and H received daily subcutaneous injections for 7 days, respectively, of 0.5 mg. ACTH in 0.1 cc. oil, 1 mg. GH in 0.1 cc. acid water, 1 mg. hydroxycorticosterone

in 0.1 cc. oil, 1 mg. GH plus 1 mg. hydroxycorticosterone in 0.2 cc. volume, 200 micrograms of epinephrine in oil, and 0.1 cc. of either acid water or oil. Since there was no statistically significant difference between the animals receiving either vehicle, these were pooled. Three hours after final injections on the seventh day, all animals were injected intravenously with approximately 3 microcuries of Au^{198} in 0.3 cc. volume, and were sacrificed by exsanguination one and one-half hours later. The spleens, livers, and lungs were removed and analyzed for Au^{198} content.

Data were analyzed by the analysis of variance, using Snedecor's F value as the test of significance (36). Individual means were compared by use of the Least Significant Difference (LSD):

$$LSD = \sqrt{s^2\left(\frac{n_1+n_2}{n_1n_2}\right)} \times t$$

where s^2 is the estimate of the error variance, n_1 and n_2 are the numbers of samples in each mean, and t is Student's "t" at the 1% level of significance, based on the degrees of freedom in s^2. Any means differing by a value equal to or greater than the LSD are significantly different at the 1% level. Unless otherwise indicated, all statements made in the text regarding differences between mean values are significant at the 1% level.

RESULTS

A. *Adrenal influences*

The values for the uptake of Au^{198} by the organs of normal and adrenalectomized rats at various times after intravenous injection of this isotope are shown in Table 1. In the normal animal, within 1 minute much of the injected radioactivity was found in the blood; however, the liver had already removed a considerable quantity of the Au^{198}. The lung at this time contained approximately 1/6 and the spleen 1/10 as much as the liver, while the thymus and lymph nodes possessed relatively small amounts of the gold. The muscle tissue was only slightly radioactive.

The same general pattern was observed in the adrenalectomized animals, immediately after injection of Au^{198}. However, the blood, lymph nodes, thymus and muscle of these animals contained relatively more radioactivity than in corresponding organs of the controls.

At 15 minutes, most of the Au^{198} had disappeared from the blood of both normal and adrenalectomized rats. The uptake by the spleen and liver was considerably increased over the values noted at one minute and the radioactivity for all other tissues was lowered.

By one to one and one-half hours, only a very small amount remained in the blood; values in the lung were reduced somewhat more; lymph node and thymus levels remained about the same, whereas values for muscle tissue were negligible. All these levels were relatively unchanged throughout the rest of the time periods observed. Moreover, the spleen and liver by this time had accepted maximal quantities of Au^{198}, and the differences between the organs of the normal and adrenalectomized animals were manifested at this time period and maintained over the subsequent time intervals.

TABLE 1. Au^{198} DISTRIBUTION IN BLOOD AND TISSUES AT VARIOUS TIMES AFTER INTRAVENOUS INJECTION (MEANS ± STANDARD ERRORS)*

Tissues	Treatment	0–1 min.	15 min.	30 min.	1 hr.	1½ hr.	2 hr.	6 hr.	24 hr.
Blood	Normal	16.6 ± 1.2	0.090 ± 0.010	0.039 ± 0.0028	—	0.027 ± 0.0031	0.031 ± 0.0037	0.021 ± 0.0010	0.024 ± 0.0006
	Adx	21.1 ± 0.65	0.039 ± 0.0023	0.040 ± 0.0037	—	0.040 ± 0.0074	0.020 ± 0.0013	0.020 ± 0.0004	0.025 ± 0.0007
Spleen	Normal	2.7 ± 0.23	5.4 ± 0.53	5.1 ± 0.45	5.2 ± 0.23	5.2 ± 0.19†	5.6 ± 0.36	6.3 ± 0.36	5.5 ± 0.47
	Adx	3.3 ± 0.30	5.9 ± 0.15	7.0 ± 0.56	6.9 ± 0.32	7.1 ± 0.30†	6.9 ± 0.59	7.9 ± 0.49	7.6 ± 0.44
Liver	Normal	32.7 ± 3.6	46.0 ± 1.6	46.5 ± 1.9	45.8 ± 1.4	45.9 ± 0.97†	46.9 ± 0.41	56.1 ± 2.4	49.0 ± 0.63
	Adx	31.1 ± 0.99	53.0 ± 1.0	53.2 ± 1.2	54.6 ± 0.88	55.8 ± 0.84†	58.0 ± 2.06	62.0 ± 1.8	55.0 ± 1.8
Lung	Normal	5.5 ± 0.49	1.20 ± 0.050	1.10 ± 0.17	—	0.71 ± 0.048‡	0.96 ± 0.069	0.81 ± 0.098	0.59 ± 0.036
	Adx	5.8 ± 0.76	0.90 ± 0.088	0.89 ± 0.066	—	0.72 ± 0.047‡	0.77 ± 0.078	0.85 ± 0.031	0.82 ± 0.098
Lymph Nodes	Normal	0.474 ± 0.025	0.383 ± 0.024	—	—	0.394 ± 0.058	—	0.422 ± 0.044	0.366 ± 0.016
	Adx	0.608 ± 0.049	0.280 ± 0.027	—	—	0.306 ± 0.019	—	0.501 ± 0.096	0.514 ± 0.057
Thymus	Normal	0.785 ± 0.072	0.026 ± 0.060	—	—	0.033 ± 0.0041	—	0.031 ± 0.0037	0.022 ± 0.0028
	Adx	0.950 ± 0.055	0.036 ± 0.0063	—	—	0.036 ± 0.0063	—	0.048 ± 0.0058	0.035 ± 0.0036
Muscle§	Normal	0.132 ± 0.000	0.0102 ± 0.0070	—	—	0.0072 ± 0.0027	—	—	—
	Adx	0.188 ± 0.016	0.0116 ± 0.0005	—	—	0.0064 ± 0.0013	—	—	—

* Figures are given as the percent of the injected Au^{198} per gram dried tissue.
All groups consisted of 7 animals except where indicated:
† 30 animals.
‡ 8 animals
§ 2 animals

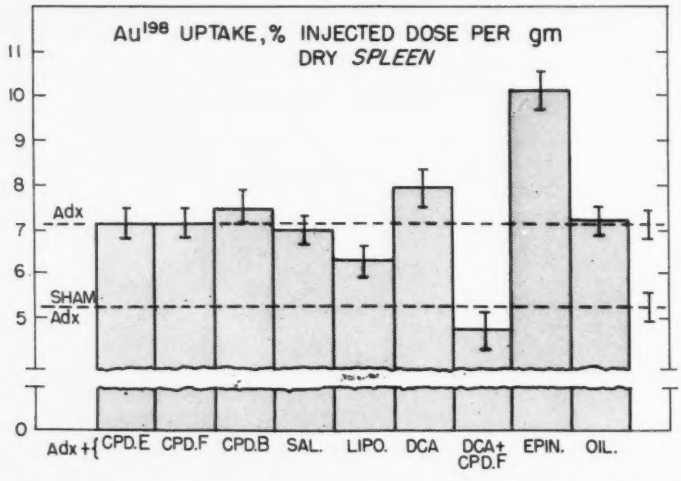

FIG. 1. Mean percent uptake of Au^{198} by spleens of adrenalectomized and sham adrenalectomized rats. Vertical lines through tops of bars represent ± 1 standard error of the mean. See text for various experimental treatments employed.

The data (Figures 1 and 2) show that adrenalectomy caused a significant increase in the uptake of Au^{198} in both spleen and liver at one and one-half hours after administration of the isotope; no changes were observed in the lung. Replacement treatment with lipo-adrenal extract, cortisone, hydroxycorticosterone, corticosterone, or a combination of DCA and hydroxycorticosterone lowered the Au^{198} concentration in the liver to normal levels; no significant alterations were noted with DCA alone. Only a combination of DCA and hydroxycorticosterone restored the quantities of Au^{198} within the spleen to normal levels. The administration of lipo-adrenal extract, cortisone, hydroxycorticosterone, corticosterone or DCA produced no significant changes in the concentrations in the spleen from the values of the operated controls. Conversely, epinephrine was observed to increase the uptake of Au^{198} by both spleen and liver significantly above that noted in the adrenalectomized controls.

B. *Hypophyseal influence*

The values for the uptake of Au^{198} by the organs of normal and hypophysectomized rats at various times after intravenous injection of this isotope are shown in Table 2. As in the case for the controls in the adrenalectomized rat series, within 1 minute after injection, much of the radioactivity was present in the blood but considerable amounts of Au^{198} were already removed by the liver. The lung at this time possessed approximately 1/4 and the spleen 1/10 as much as the liver, while the thymus and lymph nodes contained relatively small amounts of the gold. The muscle tissue showed practically no radioactivity.

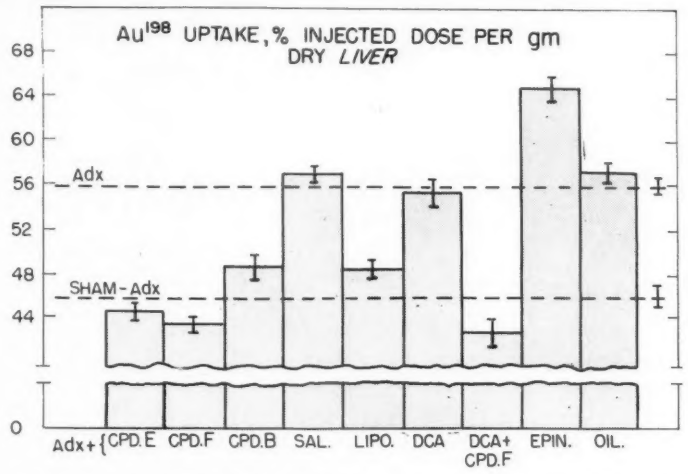

FIG. 2. Mean percent uptake of Au^{198} by livers of adrenalectomized and sham adrenalectomized rats. Vertical lines through tops of bars represent ± 1 standard error of the mean. See text for various experimental treatments employed.

A similar distribution was noted in the hypophysectomized animals immediately after injection of Au^{198}. However, all tissues except the spleen and liver were more radioactive than the corresponding organs of the controls.

At 15 minutes, most of the Au^{198} of both normal and hypophysectomized rats was removed from the blood. The uptake by both spleen and liver was greatly increased, but the radioactivity for all other tissues was lowered considerably.

TABLE 2. Au^{198} DISTRIBUTION IN BLOOD AND TISSUES AT VARIOUS TIMES AFTER INTRAVENOUS INJECTION (MEANS ± STANDARD ERRORS)*

Tissues	Treatment	0–1 min.	15 min.	30 min.	1½ hr.	6 hr.
Blood	Normal	16.6 ± 0.87	0.091 ± 0.012	0.040 ± 0.0019	0.030 ± 0.0028	0.023 ± 0.0011
	Hypx	34.0 ± 3.1	0.090 ± 0.007	0.077 ± 0.0028	0.035 ± 0.0026	0.038 ± 0.0002
Spleen	Normal	2.1 ± 0.09	6.1 ± 0.19	6.50 ± 0.35	6.15 ± 0.13†	6.26 ± 0.43
	Hypx	2.6 ± 0.18	9.6 ± 0.35	10.6 ± 0.76	11.5 ± 0.62†	13.5 ± 1.1
Liver	Normal	22.7 ± 0.84	51.1 ± 0.87	42.2 ± 0.43	43.3 ± 0.79†	46.2 ± 1.4
	Hypx	19.3 ± 1.6	69.4 ± 1.2	72.0 ± 1.8	71.4 ± 1.7†	79.0 ± 2.1
Lung	Normal	6.20 ± 0.31	1.5 ± 0.16	1.1 ± 0.14	0.70 ± 0.064‡	0.56 ± 0.037
	Hypx	13.6 ± 1.6	6.7 ± 0.06	2.7 ± 0.17	1.48 ± 0.14‡	1.60 ± 0.072
Lymph Nodes	Normal	0.384 ± 0.018	0.370 ± 0.039	0.399 ± 0.028	0.227 ± 0.027	0.224 ± 0.026
	Hypx	0.654 ± 0.050	0.440 ± 0.024	0.555 ± 0.015	0.376 ± 0.042	0.557 ± 0.077
Thymus	Normal	0.679 ± 0.061	0.040 ± 0.005	0.036 ± 0.007	0.035 ± 0.002	0.042 ± 0.006
	Hypx	1.73 ± 0.077	0.155 ± 0.016	0.231 ± 0.041	0.211 ± 0.025	0.160 ± 0.019
Muscle§	Normal	0.108 ± 0.0071	0.0101 ± 0.0021	—	0.0072 ± 0.0003	0.0083 ± 0.0006
	Hypx	0.324 ± 0.0071	0.0165 ± 0.0005	—	0.0105 ± 0.0005	0.0112 ± 0.0005

* Figures are given as the per cent of the injected Au^{198} per gram dried tissue. All groups consisted of 7 animals except where indicated:
† 15 animals.
‡ 12 animals.
§ 2 animals.

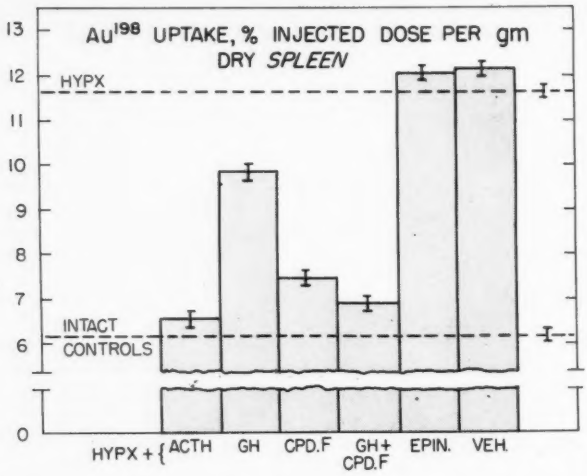

FIG. 3. Mean percent uptake of Au^{198} by spleens of hypophysectomized rats and intact controls. Vertical lines through tops of bars represent ± 1 standard error of the mean. See text for various experimental treatments employed.

By one and one-half hours, insignificant quantities were left in the blood; values in the lung and lymph nodes were reduced somewhat more; thymus levels were about the same, whereas practically no activity was observed in muscle. All these values remained relatively unchanged throughout the subsequent time periods. The spleen and liver at one and one-half hours

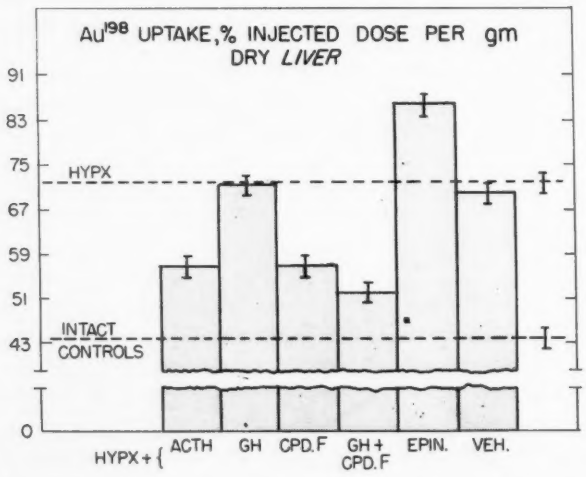

FIG. 4. Mean percent uptake of Au^{198} by livers of hypophysectomized rats and intact controls. Vertical lines through tops of bars represent ± 1 standard error of the mean. See text for various experimental treatments employed.

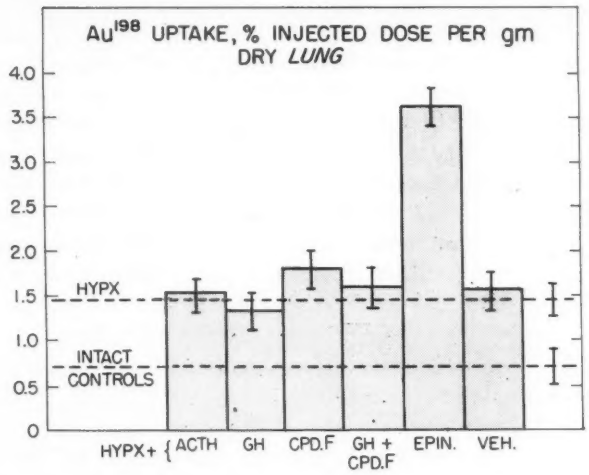

FIG. 5. Mean percent uptake of Au^{198} by lungs of hypophysectomized rats and intact controls. Vertical lines through tops of bars represent ± 1 standard error of the mean. See text for various experimental treatments employed.

had accumulated maximal quantities of Au^{198}, and the differences between the organs of the normal and hypophysectomized animals were noted by this time and persisted throughout the later time intervals examined.

The results (Figures 3, 4 and 5) indicate that hypophysectomy caused a significant increase in the uptake of Au^{198} in the spleen, liver, and lung at one and one-half hours after administration of the isotope. In the spleen, replacement treatment with GH significantly decreased the Au^{198} concentrations from the levels of the operated controls; ACTH, hydroxycorticosterone and a combination of hydroxycorticosterone and GH returned the Au^{198} concentrations to normal levels. In the liver, ACTH, hydroxycorticosterone, and a combination of hydroxycorticosterone and GH decreased the quantities of Au^{198} significantly from the values in the operated controls but not down to normal levels; GH alone had no effect. In the lung, none of these hormones had any significant influence. Epinephrine caused significant rises in the Au^{198} concentrations in the liver and lung over the operated controls, but had no action in the spleen.

The data in Table 3 demonstrate an accumulation within the liver of approximately 85–100% of the injected Au^{198}. The spleen accepted about 1% of the injected dose, whereas the other organs took up much smaller quantities. Since the radioactivity in the muscle tissue is due mainly to blood in the sample, the correction of this value for total muscle content obviously contains an inherent arithmetic error, but even this exaggerated figure contributes a negligible portion of the total recovery. Further study is necessary to determine the significance of the apparent differences be-

tween the recoveries from hypophysectomized and adrenalectomized animals.

DISCUSSION

In general, a parallel distribution of colloidal Au^{198} particles was noted in the various tissues of the intact, adrenalectomized, and hypophysectomized animals (see Tables 1 and 2). There was a rapid disappearance of the isotope from the blood, with the liver being more active than the spleen in the removal of the gold particles. Other tissues containing reticulo-endothelial elements, such as lung, thymus, lymph nodes, as well as muscle,

TABLE 3. Au^{198} DISTRIBUTION IN WHOLE ORGANS ONE AND ONE-HALF HOURS AFTER INTRAVENOUS INJECTION*

Organs	Normal	Adx	Hypx
Liver	86.43	92.41	97.55
Spleen	0.67	0.95	0.99
Lung	0.14	0.16	0.35
Lymph System	0.20	0.17	0.21
Total Muscle	0.13	0.12	0.16
Blood	0.07	0.08	0.07
Thymus	nil	nil	0.01
Total % Recovered	87.64	93.89	99.34

* Figures are given as the per cent of the injected dose.

removed much less. Other investigators have observed a similar fate of radio-gold in the blood and tissues (6, 7, 13, 14, 17, 37, 38, 39, 40, 41).

In addition, adrenalectomy resulted in a significant increase in the quantities of Au^{198} accepted by both spleen and liver. All hormonal treatments employed except DCA were effective in reducing the acceptance of the radioactive isotope by the liver to levels of sham-operated controls. However, only the combination of DCA and hydroxycorticosterone returned to normal the quantities accumulated by the spleen.

Hypophysectomy also produced an augmented uptake of Au^{198} by the spleen, liver and lung. This heightened uptake was returned to normal in the spleen by the majority of hormonal treatments employed. In the liver these factors, except GH which had no effect, decreased the quantities of Au^{198} below the values in the hypophysectomized controls but not completely to normal levels. The uptake of Au^{198} by the livers of rats which received the combination of hydroxycorticosterone and GH, however, was restored to normal levels at the 5% level of significance.

This distribution of Au^{198} is strikingly different from that previously reported for colloidal thorium dioxide (2, 3). In addition, adrenalectomy diminished the activity of the spleen below that of the liver, while adrenal cortical extract or cortisone administration to the adrenalectomized animal restored the ability of the spleen to take up normal quantities of colloidal thorium particles (1, 4, 5).

It is thus clear that the endocrine glands exercise a significant influence upon the macrophagic incorporation of colloidal radio-gold. However, unlike the situation for thorium, the hypophyseal-adrenocortical control appears to be one of inhibition rather than stimulation.

Of interest was the synergistic action exerted by the combination of certain hormones. For example, only the combination of DCA and hydroxycorticosterone restored to normal the uptake of Au^{198} by the spleen of adrenalectomized rats. Neither hormone alone, nor any of the other single hormones tested was effective. A similar action was exerted on the liver of the hypophysectomized rat; specifically, only the combination of hydroxycorticosterone and GH was effective in returning the colloidal accumulation to normal (at the 5% level of significant). Synergistic actions of this combination have also been reported effective in protecting against bacterial toxins (42), and in restoring to normal the glycolysis of bone marrow following adrenalectomy (43).

The finding that epinephrine significantly increased the uptake of Au^{198} by the spleen and liver of the adrenalectomized rat and the liver and lung of the hypophysectomized animal recalls other evidence that the medullary hormone produces an enhancement of phagocytosis independent of the adrenal (44). Mandelstamm (45) also described a proliferation of the reticulo-endothelial elements following epinephrine administration to rabbits. Other reports, however, have not been in accord with the concept that epinephrine stimulates the phagocytic mechanisms (46, 47, 48).

The results indicate a differential phagocytic response toward colloidal thorium and gold. Differences in the uptake of colloidal agents may be related, in part, to the degree of stress induced by these substances. Thus, many stresses may result in accelerated utilization of adrenal cortical hormones (49, 50). The cortical steroids, therefore, may seem to have opposing actions at different times, depending on tissue needs and dose of steroid employed. In this respect, it was demonstrated that large doses of cortisone decreased the survival of intact and adrenalectomized animals subjected to the stress of experimental pneumococcal infection, whereas smaller optimal doses of this hormone enhanced the host resistance to the infectious agent (51, 52, 53). These changes may be due, in part, to alterations in phagocytic function. Further evidence has appeared recently (54) for a stimulatory effect of small doses of cortisone on macrophagic activity.

Other factors may be responsible for the difference in phagocytic response toward colloidal thorium and gold. Thus differences in the size (6, 7, 13, 23, 47, 55, 56, 57, 58), numbers (47, 59, 60), organic coating (19, 27, 41, 61, 62), and electrostatic charge (63, 64, 65, 66, 67, 68) of these two types of particles may be important in this connection.

The increased deposition of Au^{198} in the liver and spleen of adrenalectomized or hypophysectomized animals could not be accounted for by concomitant decreases in the other reticulo-endothelial organs examined. It

was thought, therefore, that an analysis of the data on the basis of the total uptake of Au^{198} by the whole organ, rather than per gram of dry tissue, might be more informative. However, the same differences between normal, adrenalectomized and hypophysectomized animals as observed per gram dry tissue were apparent on the basis of the total uptake. The differences between the total amounts recovered from the normal and operated animals were almost entirely accounted for by quantities within the liver and spleen. The depot of Au^{198} from which the liver obtained its increased quantities in the operated animals was not clear from these studies.

SUMMARY AND CONCLUSIONS

Levels of Au^{198} in the blood decreased quickly following its intravenous administration. Adrenalectomy caused a significant increase in the uptake of Au^{198} by the spleen and liver. The lung, lymph nodes, and thymus accepted small quantities of Au^{198}, but uptake by these organs seemed unaffected by removal of the adrenal. Replacement treatment in the adrenalectomized rat with lipo-adrenal extract, cortisone, hydroxycorticosterone, corticosterone, or a combination of DCA and hydroxycorticosterone reduced the Au^{198} concentration in the liver to normal levels; no significant alterations were noted with DCA alone. Only a combination of DCA and hydroxycorticosterone restored the concentrations of Au^{198} in the spleen to normal levels. Epinephrine significantly increased Au^{198} uptake of the spleen and liver above the levels seen in adrenalectomized controls.

Hypophysectomy caused an increase in the uptake of Au^{198} by the spleen, liver, and lung. The lymph nodes and thymus of hypophysectomized animals also accepted larger quantities of Au^{198} than normal animals. Replacement treatment in the hypophysectomized rat with GH, ACTH, hydroxycorticosterone, or a combination of hydroxycorticosterone and GH significantly decreased the Au^{198} concentration in the spleen, the latter 3 hormone preparations lowering the values to normal levels. ACTH, hydroxycorticosterone, or hydroxycorticosterone and GH also significantly reduced values in the liver below those seen in operated controls; GH had no effect. None of the hormones produced any changes in the lung. Epinephrine significantly increased uptake of Au^{198} by the liver and lung over that seen in the hypophysectomized controls. The liver accumulated 80–100% of the injected Au^{198}, the spleen approximately 1%, and the other organs much smaller quantities.

The accumulation of Au^{198} and thorium dioxide by the various macrophagic elements was compared. Previous conclusions made that the pituitary-adrenal axis exerts a regulatory influence on phagocytosis remain valid. It is apparent, however, that its role is complex and may be acceleratory or inhibitory depending upon the type of colloidal particle employed and the particular reticulo-endothelial organ examined.

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THE EFFECTS OF ESTROGEN AND RELAXIN UPON THE UPTAKE OF GLYCINE-1-C^{14} BY CONNECTIVE TISSUE *IN VIVO*[1]

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AN EARLIER paper from this laboratory (4) dealt with the effects of estrogen and relaxin upon the incorporation of labeled glycine into connective tissue proteins of the guinea pig symphysis pubis *in vitro*. The administration of estradiol-17β to ovariectomized guinea pigs for one week or longer increased glycine uptake by symphyseal slices five-fold or more. Symphyseal connective tissue from relaxed animals (estrogen followed by relaxin) likewise showed elevated *in vitro* incorporation rates compared to castrate controls. Unexpectedly, relaxin alone, in the absence of prior estrogen treatment, was found to be approximately as effective as estrogen in stimulating glycine uptake. The incorporated activity was found to be present in each of the three protein fractions examined, with the largest amount in the collagen fraction.

These observations led to a study of the effects of these hormones upon the incorporation of labeled glycine into proteins of the pubic symphysis *in vivo*. In addition to the obvious desirability of correlating the results of studies of hormone action by *in vitro* and *in vivo* techniques, it was hoped that by including other tissues in the study, some clue as to a possible extra-symphyseal metabolic role of relaxin might be forthcoming.

METHODS

Eighteen guinea pigs were used in these experiments. These were animals which had been used for routine relaxin assays for some months following ovariectomy. They were retired from the assay colony at least one month before beginning further treatment. All of the animals were then injected with 2 micrograms of estradiol-17β (in 0.1 ml. sesame oil) five times weekly during one month, at which time they were divided into four groups. The control group of four animals received no further treatment until the injection of labeled glycine twelve days later. Five were continued on estradiol for another two weeks. The other two groups, after having been off estrogen for twelve days, were given relaxin subcutaneously for two and four days respectively. The relaxin was prepared from ovaries of pregnant sows according to the procedure outlined by Frieden and Hisaw (1) and contained 30 guinea pig units (GPU) per milligram. The total daily dose was 5 mg. (150 GPU), in two injections.

On the penultimate day of the experiment, each guinea pig received a subcutaneous injection of a measured amount of carboxyl-labeled glycine[2] (150,000–400,000 CPM

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[2] Obtained from Tracerlab, Inc., Boston, Mass. The specific activity was 1.3 mc./mM.

/100 gm. body weight) and was sacrificed 24 hours later. Blood was drawn by heart puncture before killing. The interpubic ligament was dissected out, and the xiphoid process, one horn of the uterus, and samples of liver and skeletal muscle, removed. The blood was allowed to clot and the serum separated. Serum and tissue samples were stored frozen until processed. There was no palpable or visible evidence of relaxation in any of the guinea pigs.

The tissue samples were minced, homogenized in 0.9% NaCl, and fractionated, as previously described (2) into water-soluble, alkali-soluble, and alkali-insoluble ("collagen") fractions. The proteins of the first two fractions were precipitated by the addition of trichloroacetic acid to 5%; each protein sample was then washed successively with acetone, a mixture of chloroform, ethanol and ether (1:2:2) and benzene, and finally plated from benzene. Only a negligible amount of alkali-insoluble material remained after fractionation of the liver sample; this was discarded. In some instances, it was necessary to combine the water-soluble protein fractions from symphysis or xiphoid tissue of two animals to get enough material for quantitative measurement of weight and radioactivity.

Serum proteins were precipiated once with 5% TCA and prepared for plating as above. After two additional precipitations with TCA, the specific activity figures were lowered by only 17%.

The precipitated and dried proteins were counted in a windowless flow counter operated in the proportional range. All counts were corrected to a uniform sample thickness of 5.0 mg./cm.^{2} and calculated specific activities (CPM/mg. protein) were further corrected to correspond to the injection of 400,000 CPM/100 gm. of body weight.

RESULTS

The selection of a suitable time interval after injection of isotopic glycine was somewhat arbitrary, since preliminary experiments with estradiol-treated guinea pigs indicated that the rate of accumulation of radioactive glycine by tissue protein varied not only between various kinds of tissues, but from one protein fraction to another within a single tissue. Thus, the specific activity of serum proteins, as well as of the water-soluble proteins of both symphysis pubis and uterus, reached a maximum within 12 hours after injection and declined steadily thereafter, whereas the activity of the alkali-soluble proteins of both tissues remained substantially constant from the 11th to the 36th hour. In contrast, the activity of the collagen fraction reached a maximum at 24 hours and diminished at 36 hours, to 40–70% of the peak value. Since our major interest was in the metabolism of the collagen fraction, a period of 24 hours was selected.

A comparison of the results for symphyseal proteins of the four groups is presented in Figure 1. The height of each bar represents the corrected specific activity of a sample from one animal; a wider bar indicates a sample in which material from two animals was combined. The average serum protein specific activity for each group is included in Figure 1 as a reference point.

It may be observed that negligible radioactivity is accumulated during 24 hours in symphyseal collagen of control animals. In estrogen-treated guinea pigs, glycine uptake during this period is increased 3–20 fold. The

administration of relaxin for only two days has an effect approximately the same as estrogen, while longer treatment with relaxin brings about an even greater increase.

Estrogen and relaxin also affect glycine uptake in the alkali-soluble protein fraction, the average increase in specific activity over that of the

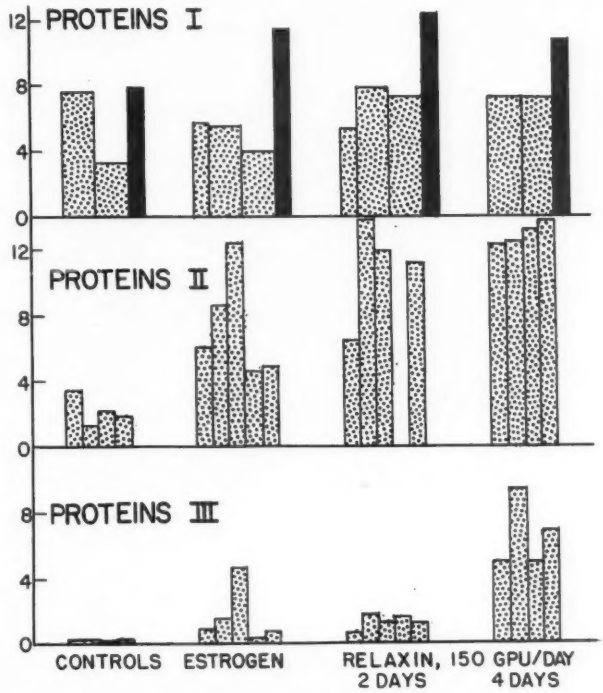

FIG. 1. Specific activities of proteins of guinea pig symphysis. Each narrow bar represents a datum for a single animal; wide bars indicate data for pooled samples (2 animals). Ordinate—CPM/mg. Proteins I: Water soluble; proteins II: alkali-soluble; proteins III: alkali-insoluble. The solid bars indicate the average serum protein specific activity for each of the four groups.

controls being 250%, 400% and 500% for the estrogen-treated and the two relaxin-treated groups, respectively. Under the conditions of this experiment, no significant differences in incorporation into water-soluble proteins could be demonstrated.

Of the other tissues examined, significant differences between control and experimental groups were found only in uterine proteins of estradiol-treated guinea pigs (Fig. 2). Relaxin has no apparent effect upon uptake of radioglycine by uterine protein, nor did either hormone affect incorporation into proteins of the xiphoid process, liver or skeletal muscle.

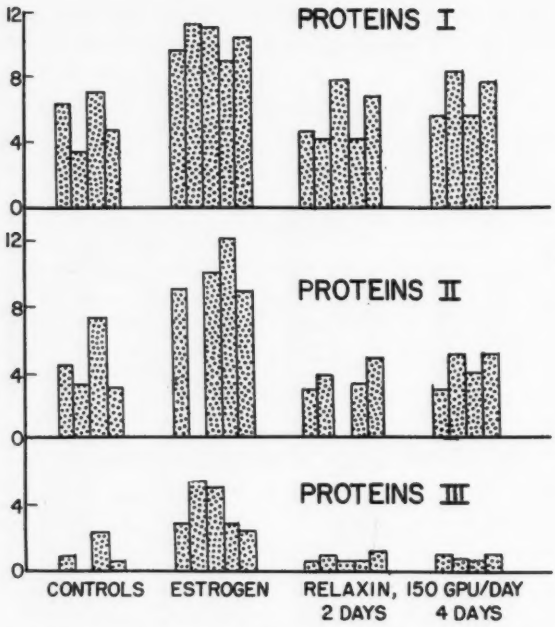

FIG. 2. Specific activities of proteins of guinea pig uterus. Ordinate: CPM/mg. Data presented as in Figure 1.

DISCUSSION

The results of this study, like those of the *in vitro* experiments reported earlier, show that relaxin exerts a significant protein anabolic effect upon the guinea pig symphysis. The one apparent discrepancy between the two studies, namely the lack of an effect upon incorporation into the water-soluble protein fraction *in vivo*, is probably due to the relatively rapid turnover rate of this component. Experiments in which a shorter time interval was used might be expected to result in demonstrable effects upon this fraction as well.

The protein anabolic action of relaxin appears to be independent of its ability to relax the guinea pig pelvis, since the former effect can be demonstrated in the absence of continuous estrogen treatment, i.e., when relaxation does not occur. Further, the data for the other tissues included in this study indicate that this effect of relaxin is confined to the symphysis pubis. Although more extensive studies might lead to a different conclusion, the apparent specificity of its action is in keeping with other observations (3) that all of the characteristic physiological effects which have been attributed to relaxin (or to relaxin-containing extracts) involve organs and tissues of the reproductive tract.

One aspect of this study which bears upon a different problem deserves

mention, namely, the significance of *in vitro* incorporation in relation to protein turnover *in vivo*. The observation that two different hormones are each capable of influencing both processes in qualitatively and quantitatively similar fashion indicates that however the two are related they are at least subject to the same regulatory mechanisms, and that inferences as to the mechanism of hormone action *in vivo* drawn from *in vitro* studies are not unwarranted.

SUMMARY

The daily administration of estradiol for two weeks increases the *in vivo* incorporation of labeled glycine into connective tissue proteins of the guinea pig symphysis pubis to three to ten times that observed in ovariectomized controls. A similar enhanced uptake is observed after only two days treatment with relaxin (150 GPU/day); extending the relaxin injections to four days brings about a further increase. Under the above conditions, estrogen also promotes incorporation into uterine proteins *in vivo*. Neither estrogen nor relaxin has any apparent effect upon incorporation into proteins of the xiphoid process, liver muscle or serum.

The significance of these results is discussed in relation to the physiology of relaxin, and the general problem of amino acid incorporation.

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BEHAVIORAL AND TISSUE RESPONSES OF MALE GUINEA PIGS TO ESTROGENS AND THE PROBLEM OF HORMONE SPECIFICITY[1,2]

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THE specificity of the relationship between gonadal hormones and the responses they elicit has been discussed by investigators interested in the action of gonadal hormones on the adult (1, 2), from the standpoint of the role of gonadal hormones on the development of fetal structures (3, 4, 5, 6), and by students of reproductive behavior (7, 8, 9). Rejection of the view that ovarian hormones stimulate only female characters and that testicular hormones stimulate only male sex characters (10, 11, 12) has been general by investigators interested in the response of adult tissues to gonadal hormones. In its place the hypothesis of an organ or tissue specificity has been proposed (1, 2, 6).

Among those who have investigated the role of gonadal hormones on sexual differentiation, there is disagreement. Moore (3, 4), noting that androgens stimulated female components to grow and that male ducts were stimulated by estrogens, rejected the concept of specificity in response to hormones during development. Burns (5, 6), on the other hand, attributed these paradoxical effects to the large quantities of hormone Moore administered rather than to any lack of specificity in response. According to Burns (5), a drastic lowering of the dose permits stimulation of all homotypic structures which ordinarily respond, while effects on heterotypic parts virtually disappear.

A number of investigators interested in reproductive behavior doubt that the relationship between hormone and response is specific. Beach (7) concludes that there are definite limits to such a concept. Eayrs (8) and Kinsey, Pomeroy, Martin and Gebhard (9) are more extreme. According to the former, "there is no truly specific relationship between any one hormone and that pattern of behavior which it facilitates." According to the latter, the designation "sex hormone" is unfortunate, they are simply

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[2] Submitted by the senior author in partial fulfillment of the requirements for the Ph.D. in Anatomy at the University of Kansas, Lawrence, Kansas.

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"among the physiologic agents which step up the general level of metabolic activity in an animal's body, including the level of its nervous function and therefore of its sexual activity."

The latter opinions do not seem to have been questioned, although there are reasons for feeling that some reservation would have been desirable. The hypothesis of non-specificity is based in part on the observations that treatment of individuals with heterologous hormones was frequently followed by a display of the heterologous behavior, or that heterologous behavior often is seen in individuals receiving the hormones commonly associated with their own sex (13, 14, 15, 16, 17). An analysis of such experiments reveals however that the responses were usually difficult to elicit, displayed at a very low level, slow and weak, rudimentary and incomplete.

Allowance does not seem to have been made for the circumstance that within the same sex a considerable degree of specificity probably exists. An atypical estrus sometimes follows the injection of an estrogen into a spayed female guinea pig, but restoration of the pattern of behavior characteristic of the individual prior to ovariectomy depends on supplementary treatment with progesterone (18). In another study of the estrous response (19) seven steroids closely related to progesterone did not substitute for this hormone. The induction of heat in estrogen-conditioned guinea pigs by desoxycorticosterone acetate (20) is suggestive of non-specificity, but only 60% of the animals were brought into heat, and among the animals which did respond, the latent period was long and heat short. Recently a number of adrenal steroids and similar compounds were tested (21). Of those which induced heat in estrogen-primed ovariectomized guinea pigs, the range of effectiveness was one-fourth to one-fortieth or less. As before, there is overlapping, but it is not a substitution. In the female rat estradiol benzoate with or without progesterone does not stimulate running behavior. On the other hand, daily injections of estrone, or the implantation of pellets of estrone restored activity to a level only slightly below that displayed prior to ovariectomy (22).

In the present study the comparative action of the hormones used in the investigations which contributed importantly to the hypothesis of non-specificity was determined. The quantities administered were within the range used in the earlier studies. The test of their action was the degree to which the patterns of behavior displayed prior to castration were maintained or restored.

MATERIAL AND METHODS

Thirty male guinea pigs approximately 150 days of age were selected from a genetically heterogeneous stock. The character of the sexual behavior of each was determined from five tests given at intervals of approximately 7 days (the preoperative interval). The animals were then castrated and placed in two groups made up in such a way that average scores were approximately equal.

One group of 17 males was used for a study of the maintenance of sexual behavior

under the influence of different hormones (maintenance-group). Beginning 8 days after castration and continuing for 16 weeks, 2 males were given 25 μg. per 100 gm. body weight of testosterone propionate[4] per day, 9 received 100 I.U. per 100 gm. body weight of estrone[5] per day, and 5 were given the same amount of α-estradiol benzoate daily. The hormones were dissolved in sesame oil and administered intraperitoneally. During the period of therapy, sexual behavior was tested weekly.

The other group was composed of 13 males used for a study of the restoration of sexual behavior following a postcastrational interval of 10 weeks when no therapy was given (restoration-group). Beginning the eleventh week and continuing for 16 weeks, 2 males were given daily injections of testosterone propionate, 5 of estrone, and 5 of α-estradiol benzoate in the amounts indicated above. Tests of these males were made every third week during the period of castrational involution and weekly during therapy.

Several controls were used. The precastrational behavior of the experimental animals was the most crucial control. Ten intact males followed in older experiments (23), and 5 intact males which were being used in a concurrent study of cryptorchidism and behavior provided data with respect to changes in behavior as animals age. The behavior of untreated castrates was obtained from older records of 7 animals (23) and from 2 males tested during this study.

The manner of testing and estimating the level of sexual behavior was in accordance with the method described elsewhere (24, 25), except that the males were not kept isolated in single cages. Instead those receiving the identical hormonal treatment were caged together. Prior to a test the male was placed alone in a cage and allowed a 20 minute adaptation period before a female in heat was placed with him. The male's behavior was then observed during a 10-minute period or until ejaculation in tests when ejaculation occurred sooner.

In order that the responses of the tissues mediating mating behavior could be compared with those of a strongly reactive genital tissue (2, 26, 27, 28) the seminal vesicles were removed and sectioned. Fixation was in Bouin's fluid, staining with Harris' hematozylin and eosin, and sectioning at 5 μ. Epithelial cell height and width of the fibromuscular layer were measured by means of ocular and stage micrometers. Averages were obtained from 25 measurements from each seminal vesicle.

The temperature of the laboratory was maintained at 70° to 75° F. The diet consisted of commercial rabbit pellets, oats, alfalfa hay, green vegetables and water. None of the animals showed any evidence of poor health following surgery or the prolonged daily injections.

RESULTS

Responsiveness as measured by tests of sexual behavior

Maintenance-group. In every group the sexual behavior score decreased during the experiment (Fig. 1). When the average scores for the last 5 weeks (11 to 16 weeks after the beginning of therapy) were compared with those during the preoperative period the decrease was 9.1% in the intact controls, 24.8% when testosterone propionate was used, 46.1% when estrone was used, 74.5% when α-estradiol benzoate was injected, and 76.0% in the single castrate.

Equally impressive differences are seen when the average quantity of

[4] Testosterone propionate was supplied by Ciba Pharmaceutical Products, Inc.

[5] Estrone and α-estradiol benzoate were supplied by the Schering Corporation, Bloomfield, N. J.

TABLE 1. MEASURES OF SEXUAL BEHAVIOR FOLLOWING TREATMENT WITH TESTOSTERONE PROPIONATE, ESTRONE, OR α-ESTRADIOL BENZOATE BEGINNING 8 DAYS AFTER CASTRATION (MAINTENANCE-GROUP), EXPRESSED IN AVERAGE PER ANIMAL PER TEST (AVE.) AND PER CENT OF ANIMALS DISPLAYING THE MEASURES OF BEHAVIOR (%)

Treatment	Number		Mounts		Intromissions		Ejaculations		Average latency of ejaculation in minutes	Average score
	Animals	Tests	Ave.	%	Ave.	%	Ave.	%		
Preoperative										
Testosterone propionate	2	10	7.8	100	7.3	100	0.6	100	7.3	10.1
Estrone	9	45	4.6	100	3.8	100	0.7	100	5.8	9.2
α-estradiol benzoate	5	25	7.2	100	5.5	100	0.6	100	8.7	9.5
Castration	1	5	4.4	100	4.4	100	1.0	100	6.3	11.0
Postoperative (1–5 weeks after beginning of therapy)										
Testosterone propionate	2	6	14.3	100	3.8	100	0.2	50	8.8	8.6
Estrone	9	27	10.5	100	1.8	100	0.1	22	9.5	7.2
α-estradiol benzoate	5	15	6.7	80	1.0	80	0.0	—	—	4.9
Castration	1	2	6.5	100	0.0	0	0.0	0	—	5.3
Postoperative (6–10 weeks after beginning of therapy)										
Testosterone propionate	2	8	13.6	100	2.5	100	0.3	100	9.7	7.5
Estrone	9	36	6.2	100	1.7	56	0.2	33	8.0	7.4
α-estradiol benzoate	5	18	1.4	80	0.0	0	0.0	0	—	2.6
Castration	1	2	0.0	0	0.0	0	0.0	0	—	2.6
Postoperative (11–16 weeks after beginning of therapy)										
Testosterone propionate	2	10	9.2	100	1.2	100	0.1	50	9.5	7.6
Estrone	9	45	6.1	89	0.5	22	0.0	0	—	4.9
α-estradiol benzoate	5	21	1.9	80	0.0	0	0.0	0	—	2.4
Castration	1	2	0.0	0	0.0	0	0.0	0	—	2.6

each measure of behavior and the average latency of ejaculation are compared (Table 1). The decrease in the percent of estrone-injected animals achieving ejaculation in the 1- to 5-week- and the 6- to 10-week-periods was large. The loss of the capacity to reach ejaculation was even more rapid in the males injected with α-estradiol benzoate. The percent achieving intromission and the average number of intromissions per animal, also decreased. As with ejaculation, the decreases were greatest in the animals given α-estradiol benzoate. The percent of mounting decreased somewhat. The smaller decrease is accounted for by the fact that this measure in the male is less affected by gonadal hormones than are intromission and ejaculation (29). Clearly, as the scores also indicate, neither estrogenic substance substituted for the androgen. It is also apparent that estrone and α-estradiol benzoate are not equivalent. After 12 weeks, the level of behavior of the males injected with α-estradiol benzoate was near that of the castrates.

Restoration-group. The results are so similar to those obtained from the maintenance-group that the graph and table corresponding to Figure 1 and Table 1 are not reproduced. The decreases in the average scores for the period 11 to 16 weeks after the beginning of hormone administration were 11.2% in the controls, 14.0% when testosterone propionate was used, 23.9% when estrone was used, 78.1% when α-estradiol was injected, and 76.9% in the castrate. Estrone therefore was not as effective as testosterone

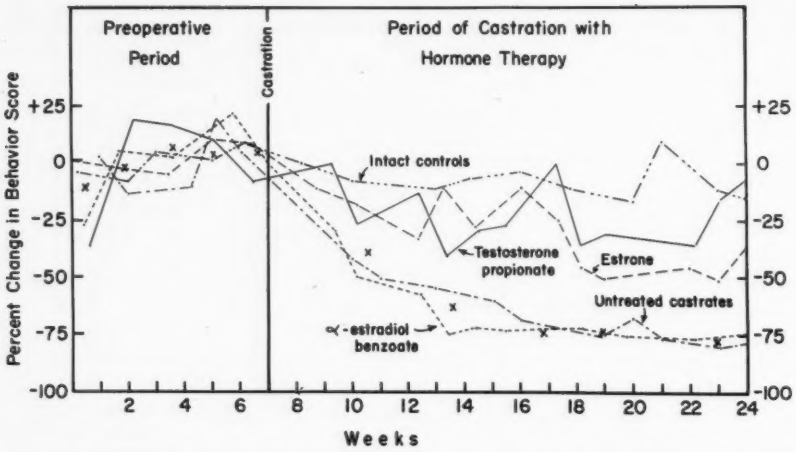


FIG. 1. Sexual behavior scores of males during the precastrational period and during maintenance after castration with testosterone propionate, estrone, and α-estradiol benzoate. A check on the average scores of the 2 castrate controls is provided by the records from 7 castrates (Xs) used in an earlier study (Grunt and Young, 1952).

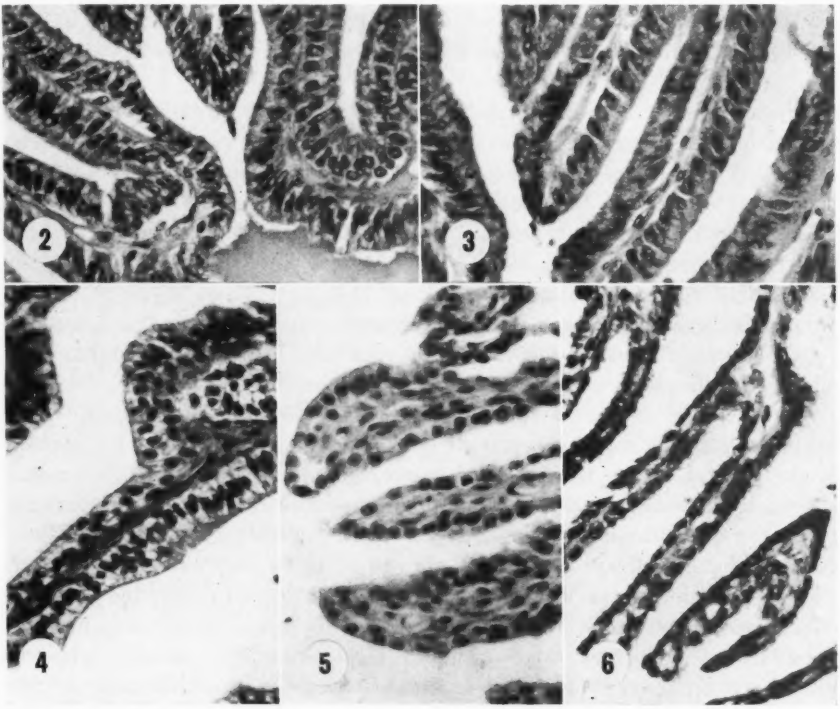
propionate, and α-estradiol benzoate had little if any action. The difference in response to the hormones is also revealed when the quantities of each measure of behavior and the average latency of ejaculation are compared. Both males injected with testosterone propionate achieved ejaculation, but only 3 of 5 of the estrone-injected males achieved intromission and none injected with α-estradiol benzoate displayed a measure of behavior higher than mounting.

Responsiveness as measured by the condition of the seminal vesicles

The reaction in the maintenance- and restoration-groups appears to have been similar. The data therefore are considered together.

An understanding of the effects of the different treatments depends on a knowledge of the action of androgenic and estrogenic substances on this organ. In the mammals which have been studied (rat, mouse, guinea pig, ground squirrel, rhesus monkey, magot) the fibromuscular layer is normally responsive to estrogens whereas the epithelium is stimulated to growth and secretory activity by androgens (28, 30, 31, 32, 33, 34, 35, 36, 37). Hyperplastic changes in the proportion of the fibromuscular layer therefore were not unexpected, but there was no indication of a specificity with respect to the action of the two estrogens. In the intact males and in those given testosterone propionate the average percent of the fibromuscular coat to the total diameter was 36.6% and 38.6%, respectively; in the castrates given estrone and α-estradiol benzoate the averages were 54.2% and 56.4%.

Examination of the epithelium, on the other hand, reveals a relationship which corresponds closely to that encountered when sexual behavior is the end point. Average cell-height is essentially similar in the intact males (17.5 μ) and in those receiving testosterone propionate (17.8 μ). It is lower in those injected with estrone (13.4 μ) and lowest in the castrates (8.1 μ) and in those given α-estradiol benzoate (9.1 μ). It is clear however that more is involved than cell-height. The appearance of the seminal vesicle epithelium from the males receiving testosterone propionate (Fig. 3) resembles that in the seminal vesicles removed from the intact males (Fig. 2). In both the epithelium is columnar, the nuclei basal and the cytoplasm secretory. Secreted material fills the lumina. The appearance of the epithelium in the animals given estrone (Fig. 4) is different, but more like that in intact or androgen-treated males than in the castrate (Fig. 6) or α-estradiol benzoate-injected males (Fig. 5). The nuclei are located basally, but the supranuclear cytoplasm appears to be devoid of secretory material and the lumina do not contain secretion-substance.



FIGS. 2–6. Seminal vesicle epithelium (×450). Fig. 2, intact male; Fig. 3, castrated male 16 weeks after daily injections with testosterone propionate; Fig. 4, after corresponding treatment with estrone; Fig. 5, after treatment with α-estradiol benzoate; Fig. 6, from castrated male.

The condition does not appear to have been described except by Wells (36) who noted that injections of estrin from 7 to 17 days into normal young male ground squirrels increased the height of epithelium in secretory tubules. The increase was due largely to a thickening of the cytoplasm, but secretion was not stored in the lumina. Elsewhere (34), a slight stimulation of the epithelium in 2 of 4 rats given estrone is noted, and Tislowitz (38) states that mitoses were seen in the seminal vesicle epithelium of castrated young mice receiving estradiol benzoate or diethylstilbestrol. For the most part, though, estrogens are reported to be without effect on the epithelium (12, 30, 32, 33, 37) or even to exert a damaging action (31).

DISCUSSION

Among investigators whose studies have been morphological, opinions with respect to the relationship between gonadal hormones and the response of the reproductive tissues have shifted from the idea of a rather specific relationship through a view favoring non-specificity to a concept of an organ or tissue specificity. With relatively unimportant reservations, the data summarized in the present study and in other work (19, 21, 22, 39, 40) are believed to justify an extension of this hypothesis to the tissues mediating mating behavior.

The specificity of the relationship between the gonadal hormones and the tissues mediating mating behavior does not appear to be an organ or tissue specificity in the sense that the organs represented in both sexes react alike to the same hormone (2). Copulatory behavior by the male is induced by androgenic substances; male-like mounting by the female, which is generally regarded as its homologue and may perhaps be mediated by the same parts of the central nervous system (41), is restored to the precastrational level by an estrogen and progesterone rather than by an androgen (40). We would recall too that the fibromuscular layer of the seminal vesicles is stimulated by estrogens rather than by androgens.

Whether the degree of specificity between the gonadal hormones and tissues mediating mating behavior is as great as that claimed for certain of the genital tissues remains to be determined. It would be surprising if there are no substances which cannot substitute completely for ovarian estrogens or testicular androgens. The synthetic estrogens, diethystilbestrol and hexestrol, are generally believed to be in this category (42), although Lipschütz and Vargas (43) direct attention to differences in their effectiveness on the endometrium. Since 1936, when estrus was first induced by estrogens and progesterone (39), estrone and α-estradiol benzoate have been assumed to act alike in conditioning the spayed female guinea pig for heat. However, in the absence of careful comparative studies, the assumption may not be justified that these estrogens are equally effective in approximating the conditioning action of endogenous estrogen.

SUMMARY

In an investigation of the specificity of the relationship between gonadal hormones and the response of the tissues mediating mating behavior in the male, the ability of testosterone propionate, estrone and α-estradiol benzoate to maintain and to restore the pattern of sexual behavior displayed prior to gonadectomy was compared. The response of the seminal vesicles was also studied.

In their action on the tissues mediating mating behavior and on the epithelium of the seminal vesicles, the three hormones in the amounts given were not equally effective. The descending order was testosterone propionate, estrone, α-estradiol benzoate. The character of the behavior and the height of the seminal vesicle epithelium in the males given α-estradiol benzoate were not greatly different from these measures of androgenic activity in castrates. The fibromuscular layer responded more strongly to the two estrogens than to the androgen and about equally to each.

Although the possibility of species- and age-differences cannot be excluded, the data are believed to justify an extension of the hypothesis of specificity from components of the genital tissues to the tissues mediating mating behavior. The specificity is to be thought of as one of individual chemical substances rather than in terms of the dichotomy, male and female hormones.

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EFFECT OF ANTIGONADOTROPHIC SERUM ON RECENT INTRASPLENIC OVARIAN IMPLANTS OF CASTRATE MICE[1]

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IF TECHNICAL error is excluded, tumors appear with consistent regularity in the laboratory rodent ovary when it is transplanted to the spleen of a castrate animal. This frequently confirmed response was first recorded by Biskind and Biskind (1), who suggested that tumors arise in ovaries transplanted to the spleen as a result of excessive and continuous gonadotrophic stimulation. In their opinion, enhanced gonadotrophic secretion follows hepatic inactivation of gonadal steroids which must pass through the liver en route to the general circulation from the ovarian implant in the spleen. The ensuing loss of the well-known inhibiting effect of ovarian hormones on the gonadotrophic secretions of the pituitary gland leads to exposure of the intrasplenic grafts to heightened gonadotrophic stimulation which eventually terminates in tumor formation.

Support for the postulation of Biskind and Biskind has been derived from widely different experimental approaches. The literature has been summarized (3, 6, 7, 8). In view of the abundant evidence suggesting the importance of the role that excessive gonadotrophic stimulation plays in the genesis of tumors in ovaries transplanted to the spleen, it seemed altogether probable that an antigonadotrophic substance would be an effective agent in limiting the growth and differentiation of ovarian splenic implants. The observations made of the effect of antigonadotrophic serum, produced in rabbits by the injection of a crude sheep pituitary extract, on young ovarian implants 30 to 70 days old at the time of autopsy are reported below.

MATERIALS AND METHODS

Pen-bred C57 Black and C57 Brown female mice 30 to 70 days old were used. Autoplastic implants of one ovary or a fragment of one ovary were made into the spleen after castration. At intervals of 1 to 28 days after surgery, subcutaneous injections of antigonadotrophic serum or control materials were begun. Doses of .2 ml. were given for 5 days each week for a period of 6 weeks.

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[2] The author wishes to acknowledge the able technical assistance of Patricia W. Palmer.

A crude sheep pituitary powder[3] extracted from whole glands according to the method of McShan and Meyer (12) was used for the development of the antigonadotrophic serum. The pituitary extract was injected into rabbits following the method described in detail elsewhere (5). Samples of serum were collected at intervals from treated rabbits and assayed for antigonadotrophic activity using a method slightly modified from that described by McShan and Meyer (12) and used by Deutsch *et al.* (4). After the appearance of antigonadotrophic activity, the serum was pooled and stored in the frozen state. Some of the serum was Seitz-filtered before injection. During the course of these experiments, the pooled antiserum was repeatedly assayed for antigonadotrophic activity. Meyer *et al.* (13) and Kupperman and Meyer (10) showed that antisera similar to those used in this study inhibit endogenous gonadotrophins in rats.

Control materials of several kinds were used. A number of animals received either normal rabbit serum or saline; some were not injected. In addition, a sheep heart antiserum, developed in rabbits by the injection of extracts of fresh sheep hearts, was given to a few animals in each experimental series. The same method of extraction was used for preparation of the sheep heart material as was utilized in the preparation of pituitary gland extracts. Procedures for the development of sheep heart antiserum in rabbits were identical to those applied when pituitary gland extracts were injected. The same number of milligrams of each of the two materials was given per injection.

Altogether 6 groups of animals were studied (Table 1). Animals in Group 1 were 30 to 40 days of age at the time of operation; the others were 50 to 70 days old. The C57 Black strain was used in Groups 1 and 2, and the C57 Brown strain in Groups 3 to 6.

At autopsy, the spleen, uterus, and thymus were weighed and the liver, kidneys, adrenals, thyroid, thymus, and pituitary glands were examined. The ovarian grafts were fixed in Bouin's solution and prepared for paraffin sectioning. Interrupted serial sections of the implant area were cut and studied. Either the hematoxylin and eosin or the periodic acid-Schiff method was used for staining.

RESULTS AND DISCUSSION

The response of the ovarian splenic implants is evaluated quantitatively in Table 1 in 4 columns. Separation of the 158 cases into 4 categories was based upon the presence or absence of follicles with large antra, on the presence or absence of cystic or bloody follicles, on the degree of stimulation of the interfollicular[4] tissue, and on the size of the implant.

As is well known, gonadotrophic stimulation is essential for the continued growth of the maturing follicle beyond the first appearance of the antrum (see Hisaw, 9, for discussion). Heightened gonadotrophic activity has also been associated with the appearance of bloody follicles in the ovary of

[3] The assistance of Dr. W. H. McShan of the Department of Zoology, University of Wisconsin, in the preparation of some of the extract used is gratefully acknowledged. According to a personal communication from Dr. McShan extracts prepared in this manner contain negligible amounts of lactogenic activity, but measurable thyrotrophic activity and, on the basis of assays of short duration, there appears to be little adrenotrophic or growth hormone activity. A portion of the extract was made from acetone dried sheep pituitary gland generously supplied by the Armour Company through the courtesy of Dr. Stanford Steelman.

[4] As used in this report, the term "interfollicular tissue" is intended to include all material not readily recognized as a follicle or a corpus luteum and thecal layers surrounding either of these ovarian bodies.

the mouse (Zondek, 15; Pfeiffer and Hooker, 14) and with cystic follicles (see Burrows, 2, for discussion). Therefore, the appearance of one or more of these enlarged stimulated follicles was taken as positive indication of gonadotrophic stimulation.

In addition, an attempt was made to evaluate the response of interfollicular tissues to treatment. As a rule, the interfollicular tissue of grafts

TABLE 1. EFFECT OF ANTIGONADOTROPHIC SERUM ON THE RESPONSE OF OVARIAN IMPLANTS TO THE SPLEEN AND ON THE WEIGHT OF VARIOUS ORGANS IN CASTRATE MICE

Group	Number animals	Treatment	Age implant first injection (da.)	Age implant autopsy (da.)	Body weight (gm.)	Uterus (mg.)	Spleen (mg.)	Thymus (mg.)	Response (number cases)			
									Maximum 1	Slight 2	None 3	No implant 4
1	7	AS[1]	1	30–32	20.5	8.2	216.7	46.1	0	0	6	1
	6	ASH[2]	1	30–32	21.5	13.8	220.0	42.6	0	2	4	0
	6	NONE	1	30–32	20.0	9.6	105.2	41.0	5*	0	0	1*
2	6	AS	1	30–31	23.1	7.4	325.3	52.6	0	0	6	0
	5	ASH	1	30–31	21.8	14.6	247.8	46.2	4**	1*	0	0
	7	NONE	1	30–31	22.3	35.9	190.0	63.2	6*	1*	0	0
3	11	AS	1	36–43	25.2	10.0	355.7	35.7	0	0	9**	2*
	12	NS[3]	1	36–43	22.1	19.7	193.1	32.6	6*	5*	0	1
	3	ASH	1	36–43	22.0	15.3	302.9	19.8	1*	1*	0	*
	8	SAL[4]	1	36–43	22.3	19.5	172.9	46.6	6**	2*	0	0
4	13	AS	5–7	44–49	26.2	9.3	273.0	48.7	0	0	9**	4**
	12	NS	5–7	44–49	24.8	15.1	241.2	48.8	8*	1	0	3
	3	ASH	5–7	44–49	26.4	13.0	208.3	42.2	3*	0	0	0
	9	SAL	5–7	44–49	25.4	18.8	196.2	51.8	5*	3*	0	1
5	7	AS	12–14	49–56	27.7	11.2	307.5*	57.6	0	0	6***	1
	7	NS	12–14	49–56	23.9	15.4	227.8	46.8	4	2*	0	1*
	2	ASH	12–14	49–56	25.2	12.1	515.0	13.8	2*	0	0	0
	4	SAL	12–14	49–56	23.9	29.7	180.7	48.0	3*	1*	0	0
6	11	AS	23–28	60–72	27.5	12.0	251.8	48.3	0	3*	7**	1*
	11	NS	23–28	60–72	26.0	16.7	238.4	42.1	9***	2*	0	0
	3	ASH	23–28	60–72	26.0	21.1	322.5	45.6	3**	0	0	0
	6	SAL	23–28	60–72	27.6	16.1	207.6	41.6	6**	0	0	0

[1] Antigonadotrophic serum.
[2] Sheep heart antiserum.
[3] Normal rabbit serum.
[4] Saline.
* Each asterisk represents one animal with adhesions between splenic surface and body wall.

from control animals was made up largely of distinctly delimited cellular aggregates often resembling in size and shape ovarian follicles with 3 to 8 layers of granulosa cells. Individual cells within the aggregates had abundant vacuolated cytoplasm with variable quantities of eosinophilic granules. Their nuclei resembled those of granulosa cells, but they were larger, and the chromatin was less compactly arranged. A few isolated periodic acid-Schiff positive "brown cells of degeneration" were generally found within the cellular clusters. Outside these distinct masses, often surrounding them or filling the interspaces between them, were strands or nodules containing 3 to 6 cells with finely vacuolated granular cytoplasm and vesicular nuclei of irregular or oval shape. The presence of this arrangement of interfollicular tissue was interpreted as evidence of gonadotrophic activity (Fig. 5).

In a few instances, the characteristic cellular clusters appeared to be pushed aside and replaced, in part, by enlarged old corpora lutea. Fre-

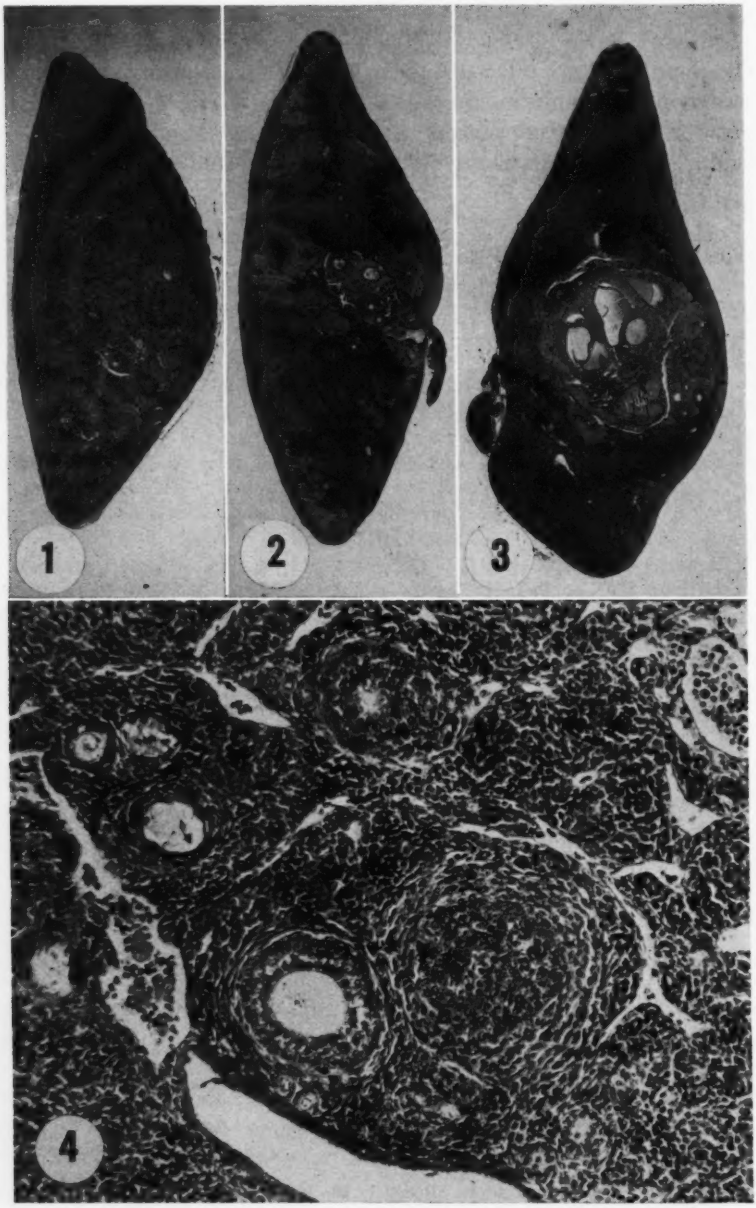

FIG. 1. Section of spleen taken through region of ovarian implant seen just below and to the right of center. ×12. This animal had received 30 injections of antigonadotrophic serum begun one week after implantation. The implant was 49 days old at autopsy. No evidence of gonadotrophic stimulation can be seen.

quently these corpora lutea had a central bloody or fibrous zone from which cords of lutein cells separated by a fine capillary net radiated. The distinct boundaries of some corpora lutea were lost, and there appeared to be an intermingling of cells from the corpus luteum with the other cell types of the interfollicular area (Fig. 8). The presence of these bodies was also interpreted as positive evidence of gonadotrophic stimulation.

A few newly formed corpora lutea were encountered throughout the various groups of experimental animals, but they were never a conspicuous part of the implant.

Finally, consideration was given to the size of the implant. All grafts visible on the surface of the spleen at autopsy showed some degree of stimulation when examined microscopically. The largest implants measured 3 to 5 mm. in diameter and often occupied a considerable portion of the cross sectional area of the spleen (Fig. 3). Implants displaying only slight evidence of stimulation on microscopic examination were often not externally visible, and they occupied a much less extensive area within the spleen (Fig. 2). None of the unstimulated or negative cases was visible externally at autopsy. Microscopically they were found to be very small (Fig. 1).

All implants showing maximal stimulation (Table 1) contained interfollicular tissue made up of the aforementioned cellular groupings. When associated with those implants containing excessively large cystic follicles, the cellular aggregates tended to be small and compressed, but usually the interfollicular tissue constituted a substantial portion of the entire graft. In the older implants, there was a notable tendency toward coalescence of the cellular clusters (Fig. 6). Grafts displaying the greatest response had follicles in all stages of development, but normal follicles with large antra or cystic follicles with or without blood in the antrum were most evident (Fig. 3). The number of normal follicles with large antra tended to be inversely proportional to the number of cystic or bloody follicles in the implant. Although not conspicuous, there was a consistent decrease with age in the number of enlarged follicular elements of all types and a concom-

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FIG. 2. Section of spleen taken through region of ovarian implant. ×12. This animal had received 30 injections of normal rabbit serum begun one day after implantation. The implant was 36 days old at autopsy. Two normal young follicles are seen in the lower right. Two degenerate follicles are in the upper field. There were no large or cystic follicles. Based on the condition of the interfollicular tissue, this case was regarded as showing a slight degree of gonadotrophic stimulation.

FIG. 3. Section of spleen taken through ovarian implant. ×12. This animal had received 30 injections of saline begun four weeks after implantation. The implant was 65 days old at autopsy. This case exemplifies maximum stimulation.

FIG. 4. Same as Figure 1. ×200. This case is typical of antiserum-treated animals. A few young follicles with several layers of granulosa cells and nests of primordial follicles can be seen. No interfollicular ovarian tissue is evident.

itant increase in interfollicular tissue. Li and Gardner (11) record the ultimate disappearance of large follicles 250 days after implantation.

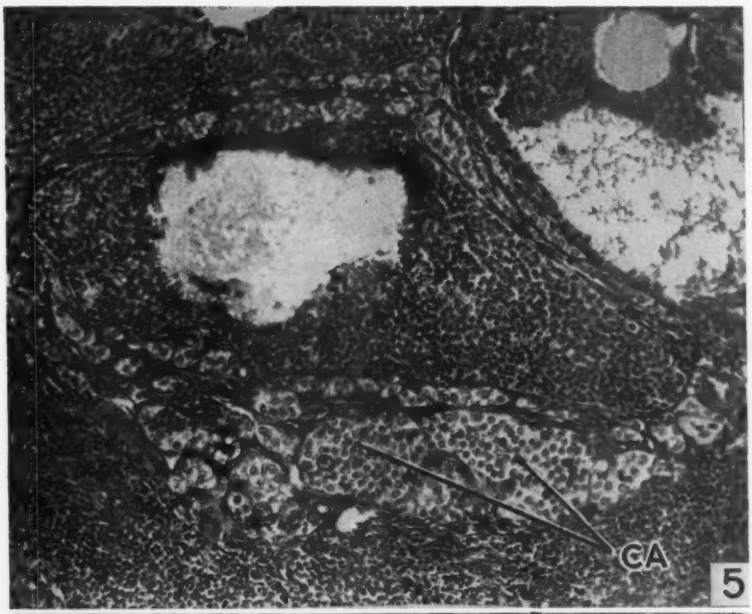
Characteristically, those intrasplenic implants indicating only slight gonadotrophic stimulation (Column 2) consisted of 1 to 3 enlarged follicles. These follicles were normal in appearance with large antra or were cystic with or without blood in the antrum. In most of these implants, no evidence of organized cellular aggregates could be found. The interfollicular tissue consisted solely of the previously mentioned strands or nodules of cells with vesicular nuclei of irregular or oval shape (Figs. 2 and 7). Three cases arbitrarily ranked among the slightly stimulated examples were made up entirely of 1 or 2 of the enlarged old corpora lutea.

Implants lacking follicles with large antra, cystic or bloody follicles, or any apparent interfollicular tissue were evidently without gonadotrophic stimulation and were placed in the negative series (Column 3). Nests of primordial follicles were abundant in the younger of these unstimulated implants, but were less numerous in older ones. A few follicles in all stages of development up to the appearance of the antrum were commonly encountered, but those consisting of 1 to 4 layers of granulosa cells were most frequently seen (Figs. 1 and 4).

The data presented in Table 1 show that the antigonadotrophic serum effectively inhibited the growth and development of ovaries transplanted to the spleen. Among 55 animals treated with antiserum, 78% showed none of the characteristic signs of gonadotrophic stimulation. On the other hand, 88% of 42 animals receiving normal rabbit serum, 96% of 40 animals receiving either normal saline or no injection, and 77% of 22 animals receiving sheep heart antiserum showed some degree of stimulation. With the few exceptions discussed below, cases in which no implant was discovered account for the remainder of the animals in each of these experimental groups. It was assumed that the missing grafts represented technical failure or that they were expelled or reabsorbed.

Four implants from animals treated with sheep heart antiserum in Group 1 were unstimulated (Table 1). Since none of these had discernible adhesions between the surface of the spleen and the body wall, no obvious explanation for the lack of stimulation of these cases is available. However, the somewhat larger uteri (Table 1) suggest the presence of some estrogen in the systemic circulatory system. In addition, three slightly stimulated implants made up of 1 or 2 follicles with large antra were found among the animals treated with antiserum in Group 6. The presence of these slightly stimulated implants in the oldest group subjected to the longest period of stimulation is probably not remarkable.

The problem of ascertaining with complete assurance that antigonadotrophic effects are mediated through an immunological interaction between the antiserum and the endogenous gonadotrophins is difficult. No entirely satisfactory resolution is reported here. However, rather than relying en-



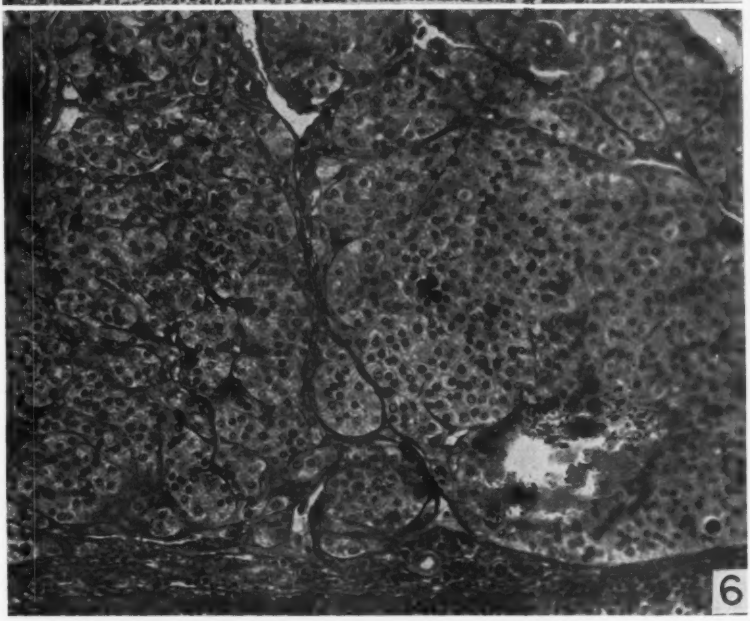


FIG. 5. Section through ovarian implant. ×200. This animal was not injected. The implant was 32 days old at autopsy. At the bottom are 2 cellular aggregates (CA) of interfollicular tissue. Partially surrounding these aggregates and between follicles small nodules made up of cells with vesicular nuclei of irregular or oval shape may be seen. A few Schiff-positive cells are evident as scattered dark cells within the cellular aggregates. This case showed maximum stimulation.

FIG. 6. Same as Figure 3. ×200. Extensive interfollicular tissue appears to be made up, in part, of fused cellular aggregates. Scattered dark cells contain Schiff-positive material.

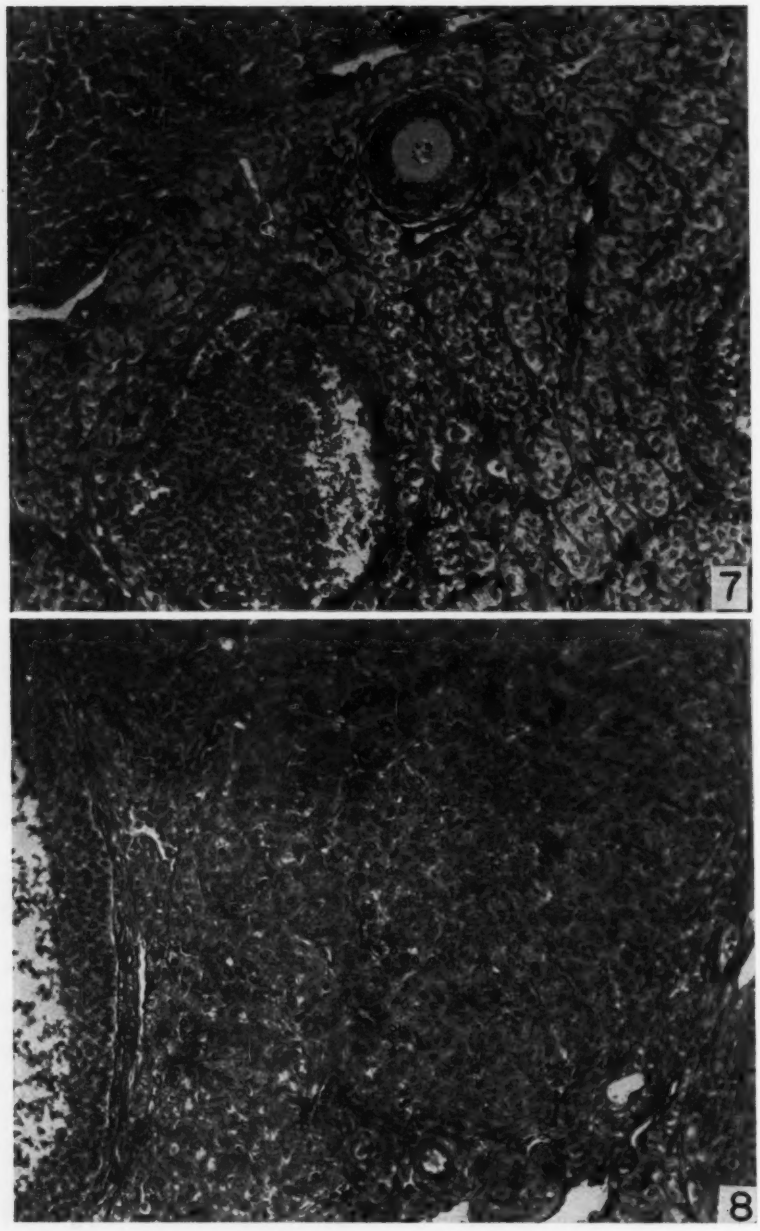


FIG. 7. Same as Figure 2. ×200. Most cases regarded as exhibiting slight evidence of gonadotrophic stimulation had interfollicular tissue as shown.

FIG. 8. Section through ovarian implant. ×200. This animal had received 30 injections of saline begun one day after implantation. The implant was 42 days old at autopsy. In this case, old corpora lutea with indistinct boundaries were a conspicuous part of the implant. This case showed slight stimulation.

tirely on uninjected or saline injected animals for control material, normal rabbit serum and sheep heart antiserum, both of which can be assumed to share many of the properties of the antigonadotrophic serum, were used. The data indicate a certain, though limited, deleterious effect of serum treatment on the development of ovarian implants into the spleen. On the other hand, the consistently smaller uteri encountered in the antiserum groups do seem to point to an endocrine response (Table 1).

Special attention was given to body, spleen, and thymus weights, since these weights may be regarded as indices of the general welfare of the experimental animals. Differences in body weight among the experimental groups were not significant. Elevated spleen weights occurred in several groups receiving antigonadotrophic or sheep heart antiserum, but they did not appear to be correlated with retardation of ovarian growth. No consistent response of the thymus was noted.

Four per cent of the total number of animals involved in these experiments showed some evidence of unfavorable response to treatment. This usually involved loss of body weight and extensive ulceration at the site of injection. However, there was no obvious correlation between poor general condition and implant response.

Cases which had adhesions between the surface of the spleen and the body wall or neighboring structures are indicated in the table. In none of these was vascularization readily apparent. In the entire series only 12 uteri weighed 25 mg. or more. It would appear that, in the young implants studied here, vascularization had not attained a degree of development sufficient to deliver significant amounts of estrogen to the general circulation.

SUMMARY AND CONCLUSION

One hundred and fifty-eight pen-bred female mice of the C57 Black and C57 Brown strains were treated with either antigonadotrophic serum (produced in rabbits against sheep pituitary gland extracts), sheep heart antiserum, normal rabbit serum, or saline at intervals of 1 to 30 days after an autoplastic ovarian transplant to the spleen.

It was found that no sign of gonadotrophic stimulation was present in 78% of the implants from animals receiving antiserum, whereas some degree of gonadotrophic stimulation was present in 88% of the implants from animals receiving one of several control solutions or no treatment at all after implantation. Cases in which no implant was discovered largely account for the remaining animals in both series. The possibility of a nonspecific toxic effect of the antiserum is discussed. The data presented in the present report are regarded as lending additional confirmation to the concept that tumors arise in ovarian grafts to the spleen of castrate animals under the influence of excessive gonadotrophic secretion.

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THE CYCLICAL CHANGES IN THE GUINEA PIG'S UTERUS, CERVIX, VAGINA AND SEXUAL SKIN, INVESTIGATED BY HISTOLOGICAL AND HISTOCHEMICAL MEANS[1]

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THIS paper describes the cyclical changes in the uterus, cervix, vagina and sexual skin of the guinea pig, investigated by histological and histochemical means. The guinea pig was selected as a favorable animal for study since it has a protracted, well-spaced reproductive cycle and the events and histological changes of its cycle have been recorded in the classical studies of Stockard and Papanicolāou (1, 2). However, the only previous study of these structures by histochemical means was limited to the vagina (3), with respect solely to glycogen, mucus and lipides. The present study includes an investigation of all of the structures listed above with reference to the presence and distribution of cytoplasmic basophilia, mucus, glycogen, lipides, protein-bound disulfide groups, alkaline glycerophosphatase, glucose-6-phosphatase and aliesterase. Thus, this paper presents much new information regarding the histochemical reactions of the guinea pig's reproductive tract. Moreover, the histochemical data of the present paper supply a background for comparison with results obtained with the electron microscope which will be reported in a subsequent communication.

MATERIALS AND METHODS

Eighteen adult female guinea pigs were examined for three months in order to ascertain their vaginal periodicity. The cycle lasted 16 days and the vagina was usually open for 3 days (proestrus, estrus, metestrus and beginning of early diestrus) and closed by a vaginal membrane (2) for 13 days. During the period of open vagina, daily smears were taken with a thin pipette; these were fixed in alcohol-ether and stained by Shorr's (4) trichrome acid method.

The animals were killed by concussion at each of the following stages of the cycle: proestrus, estrus, metestrus, early diestrus (4th day after estrus) and late diestrus (12th day after estrus).

Each reproductive tract (vagina, cervix and uterus) was dissected out and cut into 4 longitudinal strips from the vaginal orifice to the tip of the uterine horns. The strips were preserved as follows: one was fixed overnight in Zenker's acetic acid fluid, the second in Carnoy's alcohol-chloroform-acetic mixture, the third in a 4% aqueous solu-

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tion of basic lead acetate, while the fourth piece was frozen quickly at −30° C and stored at −20° C in the deep freeze.

Paraffin sections were cut at 5μ from the blocks fixed in Zenker's acetic acid fluid and the deparaffinized sections were stained for basophilic and acidophilic materials in a mixture of methylene blue ($5\times10^{-4}M$) and eosin ($5\times10^{-5}M$) in acetate and phosphate buffers at pH's 2.4, 3.5, 5.9, 8.1 and 10 (ionic strength 0.01). The blocks fixed in Carnoy's fluid were sectioned in paraffin at 5μ and stained by the chrome alum-hematoxylin and phloxine method of Gomori for the study of general histological topography, for protein-bound disulfide groups by the sulfhydryl-disulfide method of Barrnett and Seligman, (5), and for carbohydrate-containing materials by the periodic acid-Schiff (PAS) technique, with and without Harris' hematoxylin as counterstain and using saliva controls. The tissue fixed in basic lead acetate was embedded in paraffin, sectioned at 5μ, and stained for 3 hours in a 0.5% aqueous solution of toluidin blue.

The frozen blocks were sectioned at 10μ in a cryostat, mounted and dried on uncoated slides. They were stained for 5, 15 and 30 minutes for alkaline phosphatase (pH 9.3), using α-β glycerophosphate as substrate (6); for 30 minutes for glucose-6-phosphatase (7), and for 10 minutes for aliesterase using alpha naphthyl butyrate (6).

Other sections from the cryostat were fixed in 10% buffered formalin for 30 minutes, stained for 7 minutes in a saturated solution of sudan black B in 70% ethanol and then mounted in glycerogel. Control sections were first extracted for half an hour with absolute acetone at room temperature before staining them.

UTERINE ENDOMETRIUM

General histology. Upon opening the uterus, it is evident with the naked eye that its wall is thrown into a succession of circular folds and troughs. In longitudinal histological sections observed at low magnification, the endometrium appears scalloped. In the regions of the troughs, the endometrium is noticeably thicker and the glands much longer than in the alternating folds (Figs. 1, 2, 6, and 7). In the circular folds, the endometrium is thinner and the mesometrium is correspondingly thicker. The afferent and efferent blood vessels supplying the endometrium are gathered together in the mesometrial connective tissue of the folds (Figs. 2 and 7).

During estrus and metestrus, the endometrial glands are long and contain secretion and the endometrial stroma is relatively thick and vascular and the stromal cells are hypertrophied (Figs. 1, 3, 4 and 9). During late diestrus, the glands become inactive and shorter, while the stroma becomes denser, its cells smaller, and its vessels less conspicuous (Figs. 2, 5 and 11). Tissue eosinophiles are numerous during estrus and metestrus, whereas neutrophilic leukocytes predominate during diestrus. Since the granules of the neutrophiles have a noticeable affinity for acid dyes, some authors prefer to call them pseudo-eosinophiles.

The endometrial surface epithelium is pseudostratified and columnar during estrus (Fig. 3), similar but lower in metestrus (Fig. 4), and simple columnar during diestrus (Fig. 5). It possesses basally situated, cytoplasmic granules during proestrus, estrus (Fig. 3, arrow) and metestrus, while its cytoplasm becomes vacuolated during diestrus (Fig. 5, arrow). A stri-

ated border is visible on the surface during estrus and metestrus. Cilia have not been encountered at any period.

The cells of the endometrial glands are taller during estrus and metestrus (Figs. 3 and 4) than during diestrus (Fig. 5), and secretory activity is most evident during metestrus (Fig. 9). Mitoses are observed during early diestrus in the surface epithelium and deep in the glands. Terminal bars are quite evident between the cells of the surface epithelium as well as of the glands (Figs. 4 and 5, arrows).

Basophilia and acidophilia. The surface cells exhibit a diffuse cytoplasmic basophilia when stained at pH 6, which is most evident during early diestrus. The cytoplasmic granules characterizing these cells during estrus and metestrus are intensely basophilic. This basophilia becomes extinguished at pH 3.5.

In the glands, the cells are not significantly basophilic except during diestrus. The terminal bars and apical borders of the epithelial cells are acidophilic at pH 6. The stromal cells are generally acidophilic except when maximally hypertrophied, during metestrus, at which time the cytoplasm is basophilic.

Protein-bound disulfide groups. Disulfide groups stain more intensely in the surface cells than in the glandular cells. The reaction occurs generally throughout the cytoplasm and is intense in the free border and terminal bars (Figs. 12 and 13). The cytoplasmic staining occurs throughout the cycle but is most intense during proestrus and estrus. The granules present in the surface cells during estrus are also intensely positive. The stromal cells are conspicuously positive only in proestrus and estrus.

Glycogen and mucus. Traces of cytoplasmic glycogen are encountered solely in the surface epithelial cells during estrus and metestrus. The stromal cells around the bases of the glands also contain traces of glycogen at the same periods (Fig. 6). The neutrophilic leukocytes that accumulate during diestrus also display cytoplasmic glycogen (Fig. 7, arrow).

Periodic acid-Schiff positive mucus occurs on the free border of both the surface and the glandular epithelial cells (Figs. 6, 8 and 9), as well as in the apical cytoplasm of the glandular cells (Fig. 10). Mucus is most abundant in metestrus and least abundant in late diestrus (Figs. 7, 10 and 11). This epithelial mucus is weakly metachromatic with toluidin blue during estrus and metestrus and negative during diestrus. The ground substance of the underlying stroma is also faintly metachromatic during estrus and metestrus.

Lipides. Sudanophilic lipide droplets occur in all the uterine epithelial cells throughout the cycle. The droplets are very small in both the glandular and surface epithelia during proestrus. They become extremely abundant in the glandular cells during estrus and metestrus, while they remain relatively infrequent in the surface cells. During diestrus they are ex-

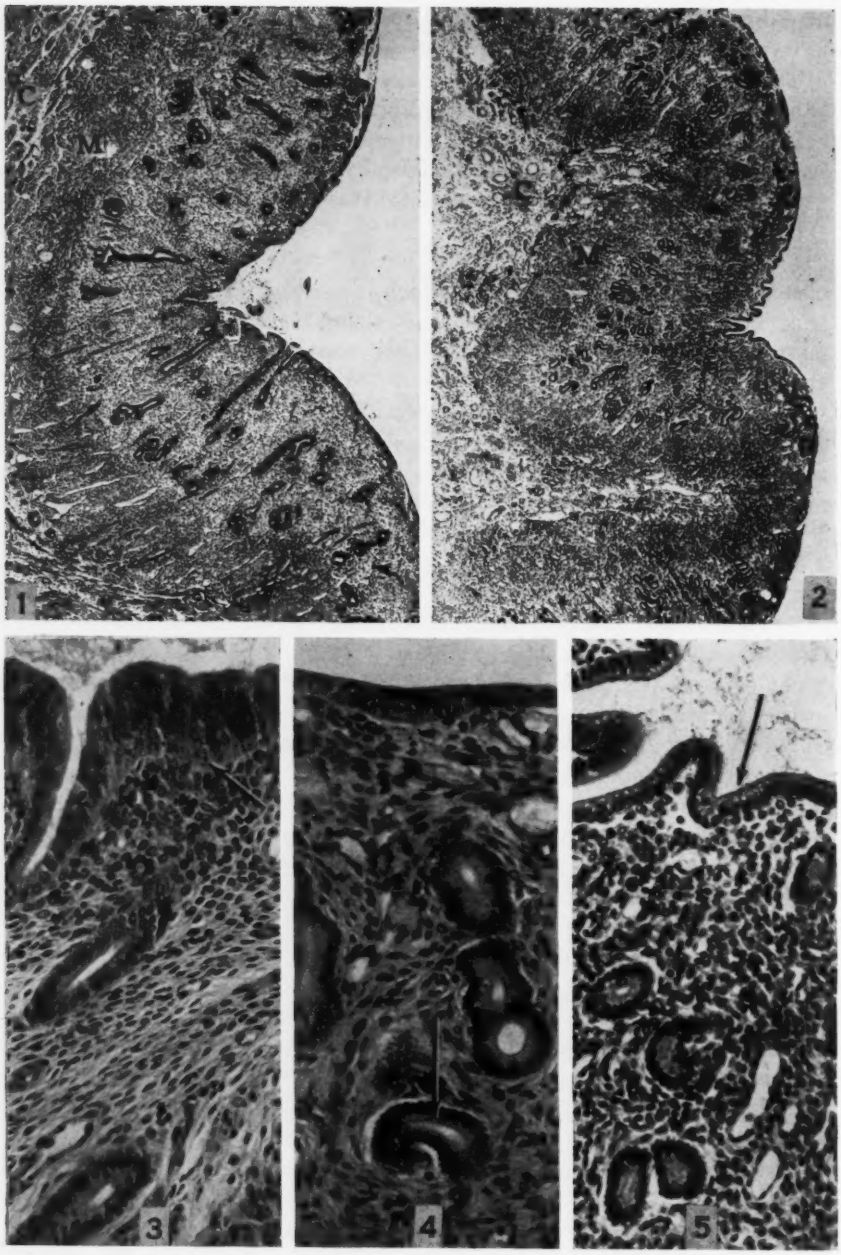

PLATE 1

tremely small and sparse in the glandular cells, but they become quite large in the surface cells (Fig. 14), thus accounting for the vacuolated appearance of the surface cells in paraffin preparations. Sudanophilic, cytoplasmic stippling, which is not affected by acetone extraction characterizes the neutrophilic leukocytes that are abundant during diestrus. A similar reaction is displayed by the larger granules of tissue eosinophiles that are also constantly present (Figs. 14 and 15, arrows).

Enzymes. Throughout the cycle, alkaline glycerophosphatase activity characterizes the free border of the glandular epithelium and is most intense during proestrus and estrus (Fig. 18). The greatest activity occurs in the middle segment of the gland (Fig. 16). During estrus, the free borders of the surface cells also sometimes stain. The cells in the underlying stroma react most intensely during proestrus (Fig. 17).

Glucose-6-phosphatase is present on the apical surfaces of the glandular cells and some in the stromal cells (Fig. 19). The reactivity occurs most conspicuously during proestrus, estrus and metestrus.

Explanation of Figures

Figs. 1 to 5 represent longitudinal sections of uterus, fixed in Carnoy's fluid, and stained by Gomori's chrome alum-hematoxylin and phloxine method.

Fig. 1. The uterine wall during metestrus, showing dilated glands and rich vascularization. E = endometrium; M = myometrium; C = connective tissue of the mesometrium. ×30.

Fig. 2. The uterine wall during late diestrus, showing smaller glands and shrunken, less well vascularized stroma. In this and the preceding figure, the alternating circular troughs and folds of the mucosa are visible, as seen in longitudinal sections. The mucosa is thicker and the glands longer in the troughs, while the mucosa is thinner and the mesometrium thicker in the folds. In the thickened mesometrial folds, the afferent and efferent blood vessels supplying the mucosa are collected, visible in the sections as wedge-shaped areas of connective tissue extending towards the uterine lumen. E = endometrium; M = myometrium; C = mesometrium. ×30.

Fig. 3. The endometrium during estrus. The surface epithelium is tall columnar and pseudostratified. Basophilic cytoplasmic granules occur in the basal portion of the cells (arrow). The glandular epithelium is a simple, low columnar one and the lumens are open. The stromal cells are spindle-shaped, except in a narrow zone below the surface epithelium. ×250.

Fig. 4. The endometrium during metestrus. The pseudostratified surface epithelium is lower than in Figure 3, and cytoplasmic granules are less abundant than at the previous stage. The glandular epithelium is somewhat taller than during estrus and the lumens are open. The arrow indicates terminal bars which are quite distinct during metestrus. The stromal cells have increased considerably in size. ×250.

Fig. 5. The endometrium during diestrus. The surface epithelium is simple columnar and contains many cytoplasmic vacuoles. The arrow indicates terminal bars, beneath which, in the apical cytoplasm of each cell, there is, as a rule, a large vacuole. The glands are shrunken, the cells compressed, and the lumens almost closed. The stromal cells are tightly packed, small, and poor in cytoplasm. ×250.

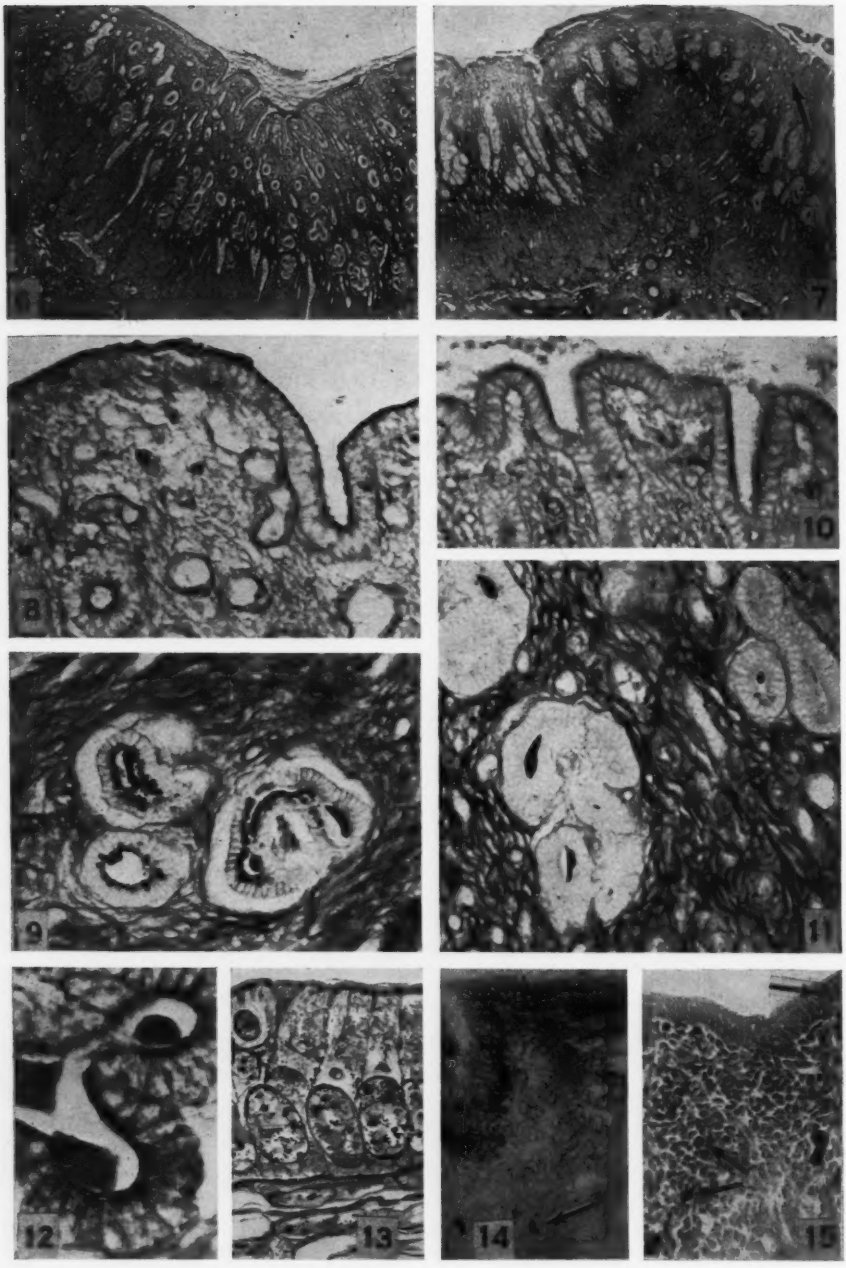

PLATE 2

Aliesterase activity characterizes the surface epithelial cells and the underlying stromal cells and is most intense during estrus and metestrus (Fig. 20).

A summary of the histochemical reactions of the uterine epithelia is presented in Table 1.

UTERINE CERVIX

General histology. The uterine cervix is characterized by a juxta-endometrial region, a middle region and a juxta-vaginal region. The middle zone, or cervix proper, consists of richly infolded cervical glands which possess

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Explanation of Figures

Figs. 6 to 11 represent longitudinal sections of uterus, fixed in Carnoy's fluid, and stained by the periodic acid-Schiff (PAS) technique.

Fig. 6. The uterine wall during metestrus. The surface epithelium exhibits an apical PAS reaction. The lumens of the glands contain secretion which is strongly stained. The stroma also gives a positive PAS reaction which is partially abolished by previous digestion in saliva, a result indicating the presence of glycogen. ×30.

Fig. 7. The uterine wall during diestrus. The surface epithelium and the endometrial glands which are almost closed are both PAS negative, excepting a few glandular lumens which contain dense, homogeneous, PAS positive mucus. The mildly positive reaction of the shrunken connective tissue is not affected by digestion with saliva. Neutrophilic leukocytes containing glycogen are numerous (arrow). ×30.

This and the preceding figure again illustrate the alternating folds and troughs of the uterine mucosa. Figure 6 shows the thickened, extremely glandular mucosa of the region of a trough whereas Figure 7 reveals the thinner mucosa and the richly vascularized, mesometrial thickening of the region of a fold.

Fig. 8. The surface epithelium during metestrus. The apical cytoplasm displays a mucoprotein reaction admixed with traces of glycogen. ×250.

Fig. 9. Endometrial glands during metestrus. The PAS reaction indicates intense mucoprotein secretory activity in the apical portions of the gland cells and in the glandular lumens. The stromal reaction was partially abolished by prior digestion with saliva. ×250.

Fig. 10. The surface epithlium during diestrus is almost PAS negative. ×250.

Fig. 11. An endometrial gland during diestrus. Mucoprotein is only present in the lumen of a few of the glands, probably representing residual secretion from the previous stage (Fig. 9). ×250.

Fig. 12. An endometrial gland during metestrus fixed in Carnoy's fluid and stained for disulfide groups. The reaction is confined almost entirely to the free border of the cells including the terminal bars, and the secretion in the lumen. ×725.

Fig. 13. A camera lucida drawing of a few cells of the previous gland showing the terminal bars intensely stained by the disulfide reagent. ×900.

Fig. 14. The endometrial surface epithelium during diestrus, showing variously large sized, sudanophilic droplets in the basal cytoplasm. The arrow points to an eosinophilic leukocyte which is intensely sudanophilic.

Fig. 15. A control section of the preceding, extracted in acetone, showing the disappearance of the sudanophilic reaction in the surface epithelium and the persistence of sudanophilia in the eosinophilic leukocytes (arrows). ×250.

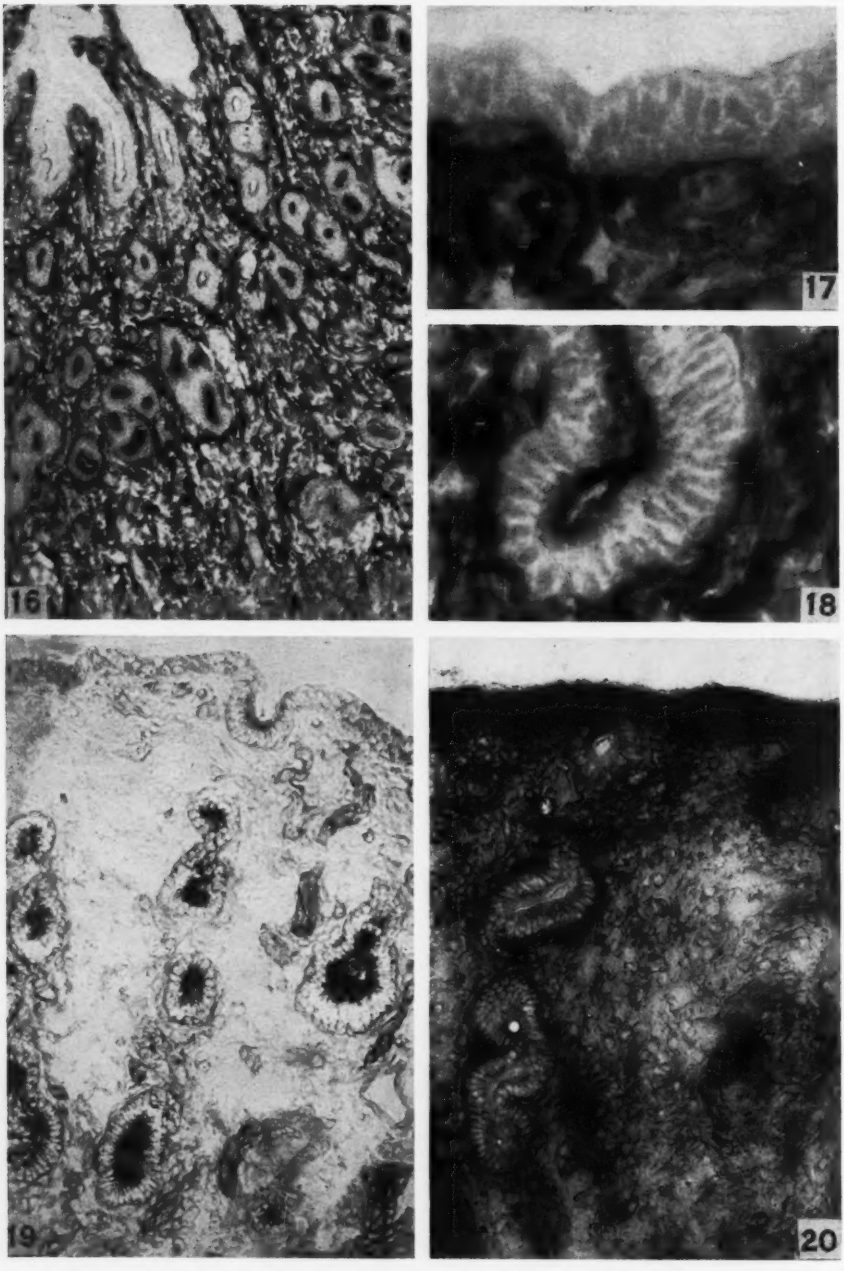

PLATE 3

TABLE 1. HISTOCHEMICAL REACTIONS OF UTERINE SURFACE EPITHELIUM (S) AND GLANDULAR EPITHELIUM (G) OF THE GUINEA PIG

	Baso-philia		Disul-fide		Glyco-gen		Mucus		Lipides		Alk. Phos.		Gluc-6-phos.		Alies-terase	
	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G
Diestrus	4	3	3	1	0	0	1	0	4*	1	0	2	0	1	2	0
Proestrus	2	1	5	3	0	0	2	1	1	1	0	5	0	3	3	0
Estrus	2	1	5	4	1	0	2	1	1	3	1	4	0	4	4	0
Metestrus	3	2	4	4	1	0	3	3	1	4	0	4	0	5	3	0

Numerals equal degree of reactions: 0—negative, 1—trace, 2—small, 3—moderate, 4—large and 5—maximal amounts.
* The lipides occur as minute droplets, except in diestrus when they are large.

two layers of epithelial cells: small cuboidal basal cells and large mucous surface cells (Fig. 23). At the uterine end of the cervix, the basal layer is continuous with the uterine surface epithelium, whereas the mucous cell layer stops abruptly (Fig. 21). Occasional patchy mucification of the epithelium occurs in the crypts of the short, coiled endometrial glands contiguous to the cervix (Fig. 23). At the vaginal end of the cervix, the basal layer becomes stratified and overlaid by several layers of mucous cells. Thus, the basal layer is continuous with the germinative and prickle cell layers of the vagina and the mucous cell layer with the vaginal mucified cells of late diestrus and proestrus (Fig. 22).

During estrus, the basal layer in the juxta-vaginal region is several cells thick and the mucous cells are extremely enlarged. The mucous cells ap-

EXPLANATION OF FIGURES

FIGS. 16–20 represent longitudinal sections of uterine endometrium during metestrus, sectioned in a cryostat.

FIG. 16. Alkaline glycerophosphatase activity is absent in the glands, excepting in the apical cytoplasm of the epithelial cells and in the secretion in the gland lumens which are both intensely stained. Enzymatic activity is greatest in the middle segment of the glands. The surface epithelium is almost negative. The stroma exhibits marked activity beneath the surface epithelium and around the glands. ×100.

FIG. 17. The surface epithelium at higher magnification, showing little alkaline phosphatase activity but a strong reaction in the subjacent stromal cells. ×350.

FIG. 18. The glandular epithelium at higher magnification, illustrating the marked alkaline phosphatase activity at the apical border of the cells and in the lumen. The stromal cells display a strong cytoplasmic reaction. ×350.

FIG. 19. Glucose-6-phosphatase. Intense activity is present at the free border of the glandular epithelium and in the secretion in the lumens of the glands. A slight reaction is noticeable in the stromal cells. ×200.

FIG. 20. Aliesterase. The surface epithelium shows intense activity whereas the glandular epithelium is almost negative. The stromal cells beneath the surface epithelium and around the glands also react intensely. ×200.

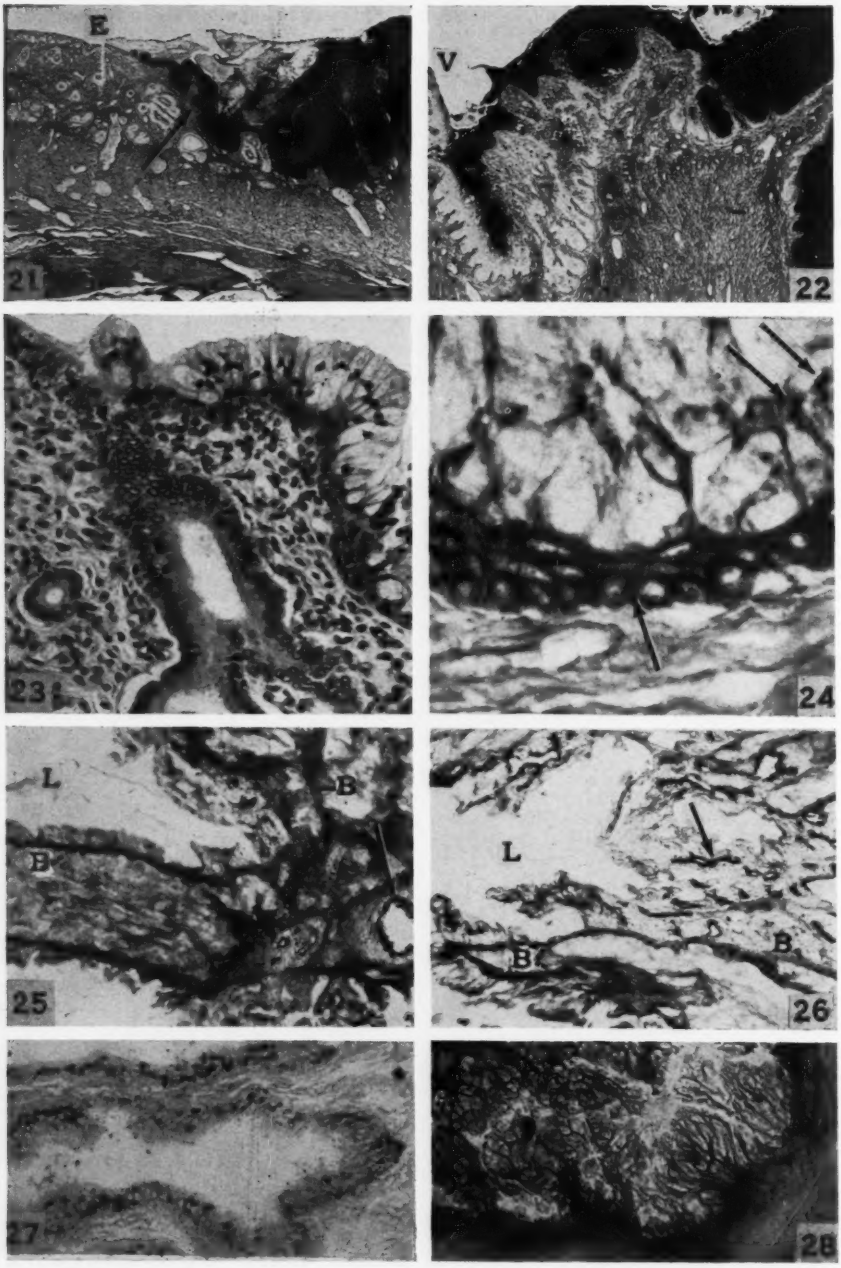

PLATE 4

pear to discharge most of their secretion during metestrus, so that during diestrus the cervical glands become much reduced in size and thickness. At this period, the well-preserved, thin basal epithelial layer and the residual mucous layer are invaded by neutrophilic leukocytes. Clear, vacuolar spaces appear also between the mucous cells. During late diestrus, some mucous cells increase in size and their cytoplasm becomes extremely foamy.

Explanation of Figures

Figs. 21 and 22 represent longitudinal sections of uterine cervix, fixed in Carnoy's fluid, and stained by the periodic acid-Schiff (PAS) technique.

Fig. 21. The juxta-endometrial region of the cervix during metestrus, showing the richly infolded cervical glands with the intensely stained mucous cell layer and the unstained narrow basal layer (arrow). The cervical mucous cells stop abruptly (E) whereas the unstained basal layer of the cervix is continuous with the surface epithelium of the uterine endometrium. ×30.

Fig. 22. The juxta-vaginal region of the cervix, during early diestrus. The cervical basal layer becomes stratified and overlaid by several layers of deeply PAS-positive mucous cells. The cervical basal cell layer is continuous with the germinative and prickle cell layers of the vagina (V) and the mucous cell layer with the vaginal mucified cells of late diestrus and proestrus. ×30.

Fig. 23. The juxta-endometrial region of the cervix during metestrus, fixed in Carnoy's fluid and stained with chrome alum-hematoxylin and phloxine. The uterine surface epithelium and a portion of an endometrial gland are visible in the center and left hand side of the photograph, whereas on the right, a row of mucous cells continuous with the cervix is visible. Below the mucous cells is an irregular layer of basal cells which is continuous, on the left, with the uterine epithelium. ×250.

Fig. 24. A cervical gland during estrus, fixed in Carnoy's fluid and stained for disulfide groups. The cytoplasm of the basal cells and the intercellular bridges (arrow below) are intensely stained. The mucous cells are negative, excepting their terminal bars which react strongly (2 arrows above). ×350.

Figs. 25 and 26 illustrate cryostat sections of cervical glands, stained for alkaline glycerophosphatase activity.

Fig. 25. A cervical gland during estrus. An intense alkaline phosphatase reaction is seen in the basal cell layer (B B), more pronounced near the lumen of the cervical canal (L) than in the crypts of the glands. The free borders of the mucous cells also react positively, but in an inverse way, in that the surface reaction is stronger in the crypts of the glands (arrow) than in the epithelium lining the cervical canal. ×200.

Fig. 26. A cervical gland during metestrus. A weaker reaction is apparent in the same places as in estrus (Fig. 25). L = lumen; B = basal layer; arrow = free border of several glandular cells. ×200.

Fig. 27. A cervical gland during diestrus. A cryostat section fixed in 10% buffered formalin and stained by sudan black B. Lipid droplets of various sizes are present in the cervical epithelium. ×200.

Fig. 28. A cervical gland during proestrus, showing aliesterase activity. There is an intense reaction in the basal cell layer, whereas the mucous cell layer is almost negative. The variable slight darkening of the mucous cells is an artifact attributable to diffusion of the reaction product. ×200.

These cells are usually pear-shaped with large, rounded, apical ends which protrude into the gland lumens. During proestrus, the epithelium becomes thicker against as more large, foamy, mucous cells arise. The intercellular bridges between the basal cells, and the terminal bars and intercellular bridges between the mucous cells, are especially conspicuous during proestrus and estrus.

Basophilia and acidophilia. Throughout the cycle, the cytoplasm of the basal cervical cells is weakly basophilic at pH 6, while the mucus of the superficial cells is distinctly basophilic at relatively low pH. In the deep portions of the cervical glands, the mucous cells are less basophilic than near the cervical canal. At pH 3.5, the mucus in the deeper portions ceases to stain, whereas the reaction in the cells near the cervical canal persists at pH 2.4. Furthermore, the basophilia of the mucus is greatest during proestrus and estrus and diminishes somewhat during diestrus. The intercellular bridges attaching adjacent mucous cells are conspicuously acidophilic at pH 6.

Protein-bound disulfide groups. The cytoplasm of the basal cells and the intercellular bridges and terminal bars between the mucous cells are intensely positive for disulfide groups. The cytoplasm of some of the foamy mucous cells in diestrus is also positive. The mucus itself does not stain for disulfides (Fig. 24).

Glycogen and mucus. No glycogen is detectable in the cervical epithelium at any phase of the cycle. A little glycogen develops in the stromal cells of the underlying lamina propria during estrus and metestrus. The mucus of the superficial cells stains intensely by the periodic acid-Schiff method. The discharge of mucus from these cells during diestrus is especially evident with this technique. The mucus stains orthochromatically with toluidin blue except during proestrus, when it is slightly metachromatic.

Lipides. Very small sudanophilic droplets occur in the cuboidal basal cells during all stages of the cycle. They are numerous during metestrus and fewer but larger during diestrus (Fig. 27). The intercellular bridges of the mucous cells are faintly sudanophilic. The neutrophilic leukocytes characterizing the diestrus cervix display an acetone-resistant sudanophilic stippling, while the tissue eosinophiles exhibit a similar reaction of their much larger cytoplasmic granules.

Enzymes. Alkaline glycerophosphatase characterizes the cytoplasm of the basal cells of the cervical glands. The reaction is relatively weak in the basal cells deep in the glands, whereas it is intense in the basal cells of the glandular outlets into the cervical canal (Fig. 25 B). Conversely, the mucous cells show a positive reaction of their apical ends which is strongest in the deep portions of the glands. In all of these sites, the reaction is strongest during proestrus and declines until early diestrus (Fig. 26). In late diestrus, the strongest reaction is confined to the cytoplasm of the neutrophilic leukocytes.

Glucose-6-phosphatase activity occurs only during estrus and is limited to the basal cells of the juxta-uterine region of the cervix.

Aliesterase activity is confined solely to the basal cells of the cervix (Fig. 28). During proestrus, estrus and metestrus, the enzymatic reaction is more intense in the juxta-vaginal region than in other parts of the cervix. During diestrus, the reactivity of the juxta-vaginal region disappears but a weak reaction persists at the juxta-endometrial region. The neutrophilic leukocytes of diestrus also react strongly.

A summary of the histochemical reactions of the cervical basal and mucous cells is given in Table 2.

TABLE 2. HISTOCHEMICAL REACTIONS OF THE BASAL (B) AND MUCOUS (M) CELLS OF THE CERVICAL EPITHELIUM OF THE GUINEA PIG

	Baso-philia		Disul-fide		Glyco-gen		Mucus		Lipides		Alk. Phos.		Gluc-6-phos.		Alies-terase	
	B	M	B	M	B	M	B	M	B	M	B	M	B	M	B	M
Diestrus	1	3	3	1	0	0	0	3	3*	0	1	0	0	0	1	0
Proestrus	2	5	5	0	0	0	0	5	1	0	5	4	0	0	3	0
Estrus	2	5	5	0	0	0	0	5	2	0	4	3	1	0	4	0
Metestrus	2	4	3	0	0	0	0	4	4	0	3	2	0	0	3	0

Numerals equal degree of reactions: 0—negative, 1—trace, 2—small, 3—moderate, 4—large and 5—maximal amounts.
* The lipides occur as minute droplets, except in diestrus when they are large.

VAGINA

General histology. The vaginal epithelium passes anteriorly into the cervical mucosa and terminates posteriorly at the vaginal orifice which is signalized by a vaginal closure membrane during diestrus and gestation. It is noteworthy that the histological and histochemical changes which characterize the phases of the estrous cycle, first appear at the vaginal orifice and subsequently spread toward the cervical end of the vagina. For simplicity's sake, the present account will begin with the vagina during late estrus, when its entire epithelium is cornified.

In late estrus, the stratified vaginal epithelium measures approximately 150μ in thickness near the cervix and 90μ near the vaginal orifice. The epithelium is only slightly indented by dermal papillae and consists of: (1) a thick basal layer composed of germinal cells, (2) a prickle cell layer, (3) a narrow transitional layer in which the nuclei are flattened and pyknotic, and (4) a thick cornified surface layer in which rarely detectable nuclei remain (Fig. 30). Only fully cornified cells appear in the vaginal smear.

During metestrus, the cornified and transitional layers are gradually desquamated leaving only the germinal and prickle cell layers. The vaginal smear consists mostly of cornified cells, transitional nucleated cells and neutrophilic leukocytes (Fig. 31). Desquamation begins at the vaginal orifice and spreads towards the cervix. A few neutrophiles accumulate in the

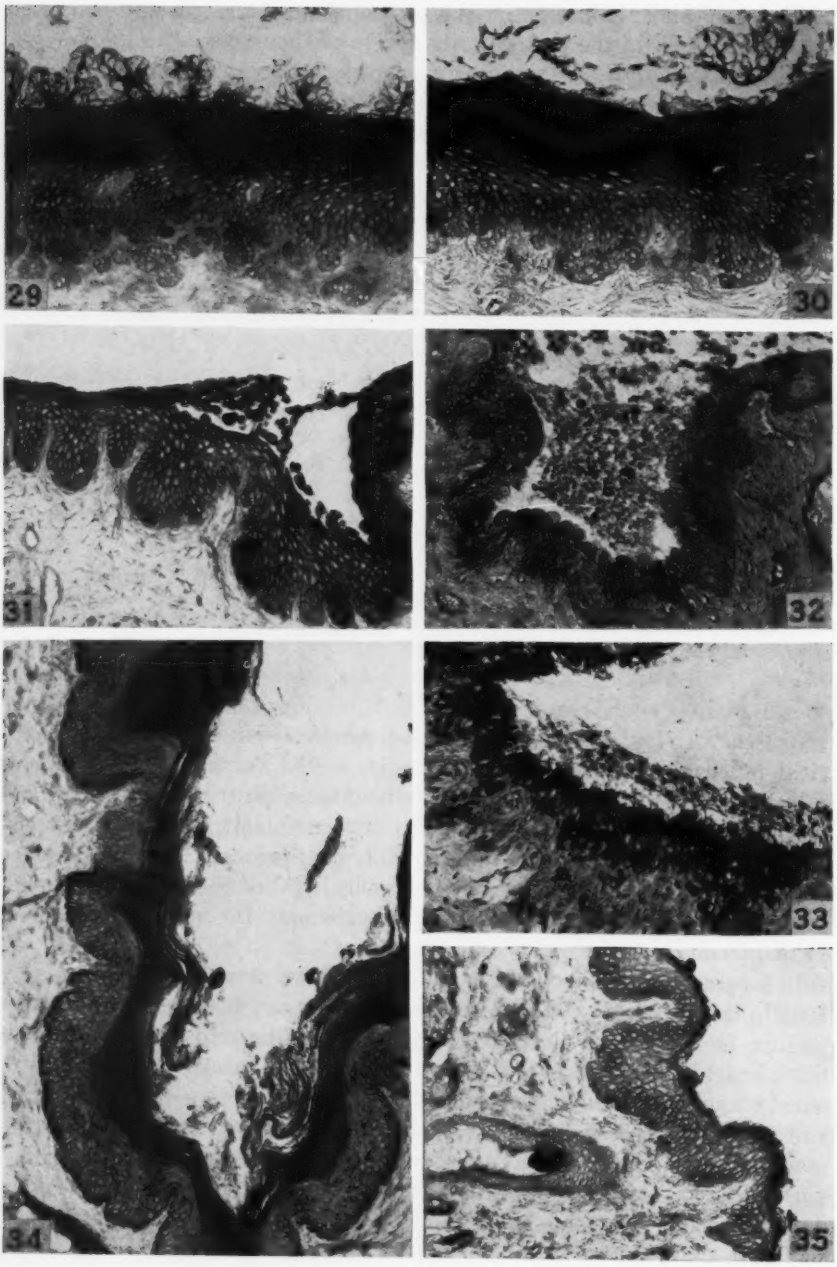

PLATE 5

lamina propria but, by the end of metestrus, most of them have migrated outward to the junction between the prickle cell layer and the residual transitional layer.

In early diestrus, the stratified epithelium has become extremely thin (20μ) at the distal end of the vagina, and is composed solely of germinal and prickle cells. The smear contains nucleated epithelial cells and numerous neutrophilic leukocytes. The latter are abundant, between the cells as well as on the surface. Nearer the cervix, the epithelium becomes thicker (40μ), although no cornified cells remain. Leukocytes are abundant here also.

In late diestrus, the epithelium near the vaginal orifice is thicker (50μ) than in the previous stage. The surface cells have become swollen and foamy in appearance, due to the accumulation of mucus (Figs. 32 and 33). This process extends towards the cervix. The epithelium now consists of three layers, namely the basal cell layer, the prickle cell layer and a mucified cell layer. Infiltration of leukocytes persists (Fig. 39). Vaginal smears at this stage consist predominantly of leukocytes, with a small representation of surface cells.

Explanation of Figures

Figs. 29 to 32 represent longitudinal sections of the proximal part of the vagina, fixed in Carnoy's fluid and stained for disulfide groups.

Fig. 29. The vagina from proestrus. The broad epithelium shows an intensely positive disulfide reaction of the transitional and early cornified layers and a moderately intense reaction of the basal layers. The mucous cells on the surface of the vagina at this stage are negative except for the cell membranes and intercellular bridges. ×125.

Fig. 30. The vagina from estrus. The entire cornified layer reacts intensely and homogeneously for disulfide groups. The reaction is much weaker in the germinative and basal cell layers. Desquamated mucous cells are visible in the vaginal lumen. ×125.

Fig. 31. The vagina from metestrus. A moderate reaction is present in the entire epithelium. The desquamating transitional cells exhibit the strongest reaction. ×125.

Fig. 32. The vagina from diestrus. A mild reaction is present in the cytoplasm of the mucous cells but none in the mucus itself. In the prickle cell and basal layers the reaction is weak and is confined mostly to the cell membranes. ×125.

Fig. 33. The vagina from diestrus, illustrating the broad, outer layer of mucous cells, deeply stained by the periodic acid-Schiff reagent after pretreatment of the section with saliva. ×200.

Figs. 34 and 35 represent longitudinal sections of the sexual skin, fixed in Carnoy's fluid and stained for disulfide groups.

Fig. 34. The sexual skin from estrus. The broad cornified layer reacts intensely for disulfide groups. Melanin occurs throughout the entire thickness of the epithelium and is responsible in large measure for the dark appearance of the inner layers of the epidermis. ×125.

Fig. 35. The sexual skin from diestrus. In this phase the skin is thinner, because of the desquamation of the cornified layer. The disulfide reaction is weaker and melanin is confined to the basal cell layer. ×125.

In proestrus, the stratified epithelium reaches its greatest thickness, consisting of germinal cells, a thick prickle cell layer and an equally thick superficial layer composed of an inner zone of flattened, cornified cells and an outer zone of mucous cells. The latter undergo desquamation in clusters (Fig. 29). The leukocytes have disappeared. The smear now consists of clusters of mucous epithelial cells with pyknotic nuclei and foamy cytoplasm.

In early estrus the clusters of mucified cells and the outermost cornified cells are usually shed as a membrane. These form the major constituents of the smear. The epithelium therefore, becomes thinner and is composed of the three layers characteristic of late estrus.

Basophilia and acidophilia. During metestrus and diestrus, the cytoplasm of the basal and prickle cells contains basophilic material (pH 6) surrounding the nucleus, while the peripheral cytoplasm is weakly acidophilic. During the rest of the cycle, the cytoplasm of the basal and prickle cells as well as their intercellular bridges, is acidophilic below pH 8.1. The mucus in the mucified cells of the outer layer is basophilic down to pH 3.5.

Protein-bound disulfide groups. During estrus, the entire cells of the cornified layer throughout the vaginal epithelium react intensely and homogeneously for disulfide groups (Fig. 30). The basal nucleated layers are less deeply stained. In the latter, the reactive material occurs principally in the peripheral cytoplasm of the cells and in the "intercellular bridges." During metestrus, the reaction is strong in the cytoplasm of the transitional layer but is weaker in the prickle cell and germinal cell layers (Fig. 31). In diestrus, the response is intensely positive in the cytoplasm of the mucous cells but negative in the mucus (Fig. 32). In the two inner layers it is weak and confined to the cell membranes. In proestrus, it becomes very intense in the transitional and cornified layers and moderately intense in the more basal cells and negative in the mucous cells (Fig. 29).

Glycogen and mucus. Intraepithelial glycogen occurs only during proestrus and estrus in the cytoplasm of the prickle cell layer. During metestrus and diestrus it is present solely in neutrophilic leukocytes. Mucus appears in the cells of the superficial layer during late diestrus (Fig. 33) and proestrus, as well as in the shed mucified membrane of early estrus. This mucus is weakly metachromatic with toluidin blue. Following staining with toluidin blue, the cytoplasm of the basal cells is faintly metachromatic during proestrus and estrus but is orthochromatic at the other stages. The cytoplasm of the cornified cells stains a blue-green rather than the orthochromatic color.

Lipides. During estrus, small sudanophilic droplets are detectable in the epithelial cells. These droplets decline during metestrus and disappear during diestrus. The desquamated mucous cells of proestrus and early estrus contain relatively large droplets. The droplets at all stages are re-

moved by pretreatment with acetone. The intercellular bridges between the prickle cells stain weakly. In addition, the granules of the neutrophilic leukocytes and tissue eosinophils in the connective tissue are sudanophilic but these reactions are not abolished by extraction with acetone as is that in the epithelial cells.

Enzymes. Alkaline glycerophosphatase is present in the vaginal epithelium during proestrus, estrus and metestrus. The reaction is most intense in the germinal basal layer, weaker in the prickle cell layer and essentially negative in the desquamative layer (Fig. 38). Leukocytes in the lamina propria also react.

Glucose-6-phosphatase is present during proestrus and early estrus only and is limited to the prickle cell layer and to the walls of the capillaries of the lamina propria (Fig. 41).

Aliesterase occurs in some portions of the vaginal epithelium at all stages of the cycle. During estrus, the reaction is intensely positive in the basal half of the vaginal epithelium and moderate in the cornified layers. In metestrus, the reactivity has diminished in the basal and prickle cell layers and, during diestrus, reactivity is limited to the mucous cells (Fig. 37, arrow). In proestrus, reactivity reappears throughout the epithelium, but is most intense in the mucous layer (Fig. 36).

The histochemical reactions of the several layers of the vaginal epithelium are summarized in Table 3.

THE SEXUAL SKIN

The vagina passes into the shallow vestibule which forms the external genital outlet. The vestibule is lined by epidermis which possesses hairs and is continuous with the skin surrounding the genital orifice.

The epidermis of the genital outlet differs in its histological appearance and histochemical reactions from the vaginal mucosa and it undergoes definite cyclical changes with respect to the estrous cycle. Thus, it seems in effect to represent "sexual skin" as known in monkeys but hitherto unreported for rodents. There are, for example, noticeable cyclical changes with respect to the thickness of the epidermis and the amounts of keratohyalin

TABLE 3. HISTOCHEMICAL REACTIONS OF THE VAGINAL EPITHELIUM OF THE GUINEA PIG (B = BASAL AND PRICKLE CELLS, M = MUCOUS CELLS, C = CORNIFIED AND TRANSITIONAL CELLS)

	Basophilia			Disulfide			Glycogen			Mucus			Lipides			Alk. Phos.			Gluc-6-phos.			Alicsterase		
	B	M	C	B	M	C	B	M	C	B	M	C	B	M	C	B	M	C	B	M	C	B	M	C
Diestrus	2	3		1	3		0	0		0	3		0	0		1	0		0	0		0	3	
Proestrus	1	5		3	1	4	3	0	0	0	5	0	1	3*	0	5	0	0	3	0	0	4	3	0
Estrus	1	—	0	4	—	5	1	—	0	0	—	0	3	—	4*	5		0	1	—	0	5	—	3
Metestrus	2	—	0	3	—	4	0	—	0	0	—	0	2	—	2	4		0	0	—	0	4	—	2

Numerals equal degree of reactions: 0 = negative; 1 = trace; 2 = small; 3 = moderate; 4 = large and 5 = maximal amounts.

* The lipides occur as minute droplets, except in the desquamated mucous cells during proestrus and in the desquamated cornified cells during estrus, when they are much larger.

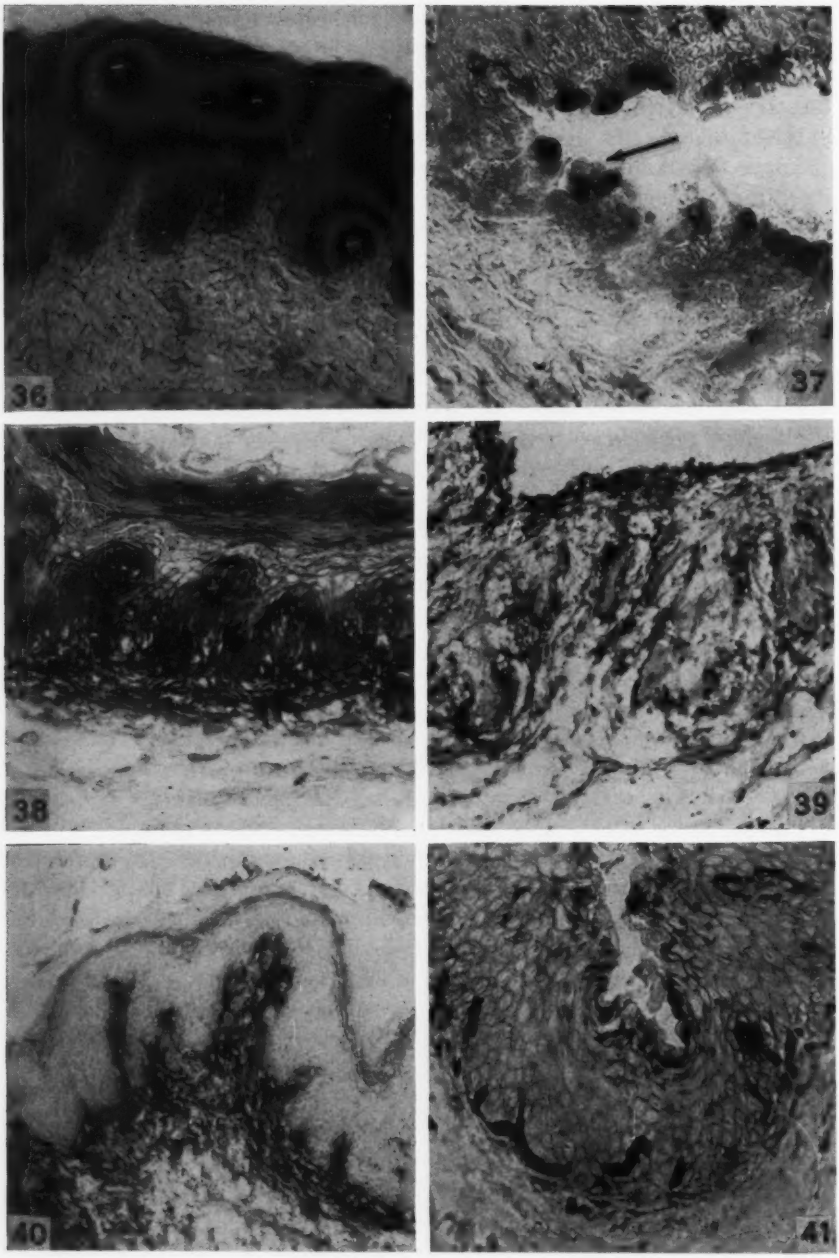

PLATE 6

and melanin, which differ from the changes in the vaginal epithalium.

In late estrus, the epidermis of the sexual skin is relatively thick, melanin appears to be present throughout the stratified epithelium (Fig. 34) and keratohyaline granules are abundant in the transitional layer (Fig. 40). During metestrus, melanin apparently diminishes and becomes restricted solely to the basal layer of the epithelium.

In early diestrus, no appreciable changes take place, but by late diestrus, the sexual skin has become thinner, melanin has disappeared except from the basal layer and keratohyaline granules are no longer visible (Fig. 35).

In proestrus, no noticeable change occurs, but in early estrus the sexual skin becomes thicker again, melanin reappears throughout the stratified epithelium and keratohyaline granules reaccumulate in the transitional layer.

The sexual skin displays a faint reaction for glycogen during proestrus and estrus which is confined to the prickle cell layer.

In contrast to the vagina, the sexual skin throughout the cycle exhibits neither alkaline glycerophosphatase (Fig. 40) nor glucose-6-phosphatase activity. In the narrow zone of transition between the vagina and the sexual skin, a reaction for alkaline glycerophosphatase is solely present in the basal cell layer. A positive reaction, in the otherwise negative skin, is visible in occasional neutrophilic leukocytes, present especially during

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Explanation of Figures

Figs. 36 to 41 represent longitudinal sections of the proximal part of the vagina or of the sexual skin, sectioned in a cryostat and stained for various enzymes.

Fig. 36. The vagina during proestrus, illustrating the activity of aliesterase. A positive reaction occurs throughout the epithelium, but it is most intense in the cytoplasm of the mucous cells. ×200.

Fig. 37. The vagina during diestrus, illustrating the activity of aliesterase. In contrast to the preceding, reactivity is limited to the cytoplasm of the mucous cells, in which the mucus itself is negative (arrow). ×200.

Fig. 38. The vagina during estrus, illustrating the activity of alkaline glycerophosphatase. An intense reaction is present in the basal epithelial layer and a weak reaction in the prickle cell, transitional and cornified layers. The lamina propria beneath the vaginal epithelium reacts intensely. ×200.

Fig. 39. The vagina during diestrus, illustrating the activity of alkaline phosphatase. The vaginal epithelium and lamina propria are almost negative, the scattered punctate reaction being the result of a strong cytoplasmic response of neutrophilic leukocytes. ×200.

Fig. 40. The sexual skin during estrus, illustrating alkaline glycerophosphatase activity. From the same section as Figure 38. The epidermis is negative, but the dermal connective tissue is strongly active. ×200.

Fig. 41. The vagina during estrus, illustrating the activity of glucose-6-phosphatase. There is an intense reaction in the walls of the capillaries of the lamina propria as well as a faint reaction which outlines the cell boundaries of the prickle cell layer. ×200.

diestrus. In contrast also to the intense reaction observed in the vagina, aliesterase is essentially negative in the skin, excepting a faint reaction in the basal layer.

DISCUSSION

Retterer (8) observed regular changes in the vaginal mucosa of non-pregnant guinea pigs. Loeb (9, 10, 11), from an investigation of ovulation and the formation of corpora lutea in the guinea pig, concluded that these events recur at intervals of about 21 days. Stockard and Papanicolaou (1) described an oestrus rhythm consisting of four stages and they correlated the vaginal smear with the histology of the vagina, endometrium and ovary.

Long (12) and Long and Evans (13) described a proestrus period in the rat's vaginal epithelium, which Stockard and Papanicolaou had not described in the guinea pig. Selle (14) restudied the guinea pig vagina and was able to distinguish a typical proestrus period corresponding to that of the rat, and his observations were confirmed by Young (15).

Morau (16) first reported mucus in the vaginal epithelium of the rat, and Barrington (17) stained it selectively with Mayer's mucicarmine. Courrier (18) used that stain in his classical study of mucification of the vaginal epithelium. Cyclical changes of glycogen, mucus and lipides in the vaginal epithelium of the guinea pig were first observed by Tribby (3). He reported that the intracellular mucoid was maximal during proestrus and was restricted to the superficial epithelial cells. The intracellular glycogen also reached a maximum concentration during the proestrus stage and was present in both the superficial and prickle cell zones. Intracellular lipides were said to be most abundant during proestrus and estrus in the superficial, transitional and prickle cell zones. Tribby's study appears to be the only previous one in which histochemical techniques have been applied to the guinea pig's reproductive tract. However, his study was confined solely to the vagina, whereas ours includes the uterine cervix, endometrium and "sexual skin." Moreover, his investigation was limited to observations on glycogen, mucus and lipides, whereas ours, in addition to those substances, includes cytoplasmic basophilia, protein-bound disulfide groups, alkaline glycerophosphatase, glucose-6-phosphatase and aliesterase.

The presence of basophilic granules and diffuse basophilia in the endometrial surface epithelium can be related principally to cytoplasmic ribonucleoprotein. The binding of basic dye down to pH 3.5 seems to support this assumption, in addition to which, the basophilic substance in these cells has been shown to be digested by ribonuclease (19). The basophilia of the cervical epithelium is largely confined to the mucous cells in which the extinction of staining with basic dyes at pH 2.4, a strong PAS reaction

and a negative reaction for disulfide groups suggests the presence of acid mucopolysaccharide. The cells of the basal and prickle cell layers of the vaginal epithelium display some perinuclear basophilia during metestrus and diestrus, which diminishes greatly during estrus. Acidophilia, which characterizes the peripheral cytoplasm and the intercellular bridges of these cells at all stages, is associated with a similarly localized, positive disulfide reaction.

During metestrus, the secretory activity of the endometrial glands is revealed by a strong periodic acid-Schiff reaction of both the glandular epithelium and secretion. During late diestrus, a few glands still contain intensely PAS positive mucus in their lumens, but the corresponding glandular cells are completely devoid of any stainable intracellular precursors indicative of secretory activity. It is concluded from this that the material in the lumen is a remnant of the previous secretory stage. The nature of this residual secretion in the glands is not entirely clear but its PAS reaction which is resistant to salivary digestion, its basophilic staining which disappears between pH 3.5 and 5.9, its mild metachromasia and its positive disulfide reaction, suggest that it may be a mixture of mucus and of nucleoprotein derived from disintegrating cells.

It is of considerable interest that the epithelial changes during the cycle do not occur synchronously in all parts of the reproductive tract but in waves that begin at the distal end and spread proximally. Thus the elaboration of mucus appears to start near the vaginal orifice in late diestrus and to reach the upper end of the vagina during proestrus. Similarly, the maximal accumulation of mucus in the cervical epithelium occurs during estrus, whereas the uterine epithelia do not become maximally laden with mucus until metestrus, a correlation which has never been reported before.

Similarly, cornification of the vaginal epithelium begins at the vaginal orifice at the end of proestrus but does not become fully developed in the juxta-cervical vaginal region until the end of estrus. This pattern of vaginal cornification was observed by Stockhard and Papanicolaou (1). The subsequent desquamation of the cornified layer proceeds in the same manner.

The finding of glycogen in the vaginal prickle cell layer during proestrus and early estrus confirms the observations of Tribby (3). Glycogen is absent at all times from the cervical epithelium and nearly so from the endometrial epithelium. During metestrus, the stromal cells present a weakly positive glycogen reaction. A correlation between glycogen and glucose-6-phosphatase activity is clearly apparent in the prickle cell layer of the vagina. In the endometrial stroma, also, a correlation is clearly evident, but at the level of the endometrial glands there is no correlation, glucose-6-phosphatase activity being present in the absence of glycogen.

Lipides are found during the entire cycle in the endometrial, cervical, and vaginal epithelia. Two types of sudanophilic droplets are distinguisha-

ble, very small droplets which are usually numerous, and moderately large droplets which are less numerous. The former are most abundant in the vaginal epithelium during proestrus and estrus, in the basal layer of the cervical epithelium during estrus, and in the uterine glandular and surface epithelia during metestrus. The larger droplets which occur in the desquamating mucous and cornified layers of the vagina during proestrus and estrus, and in the cervical and surface uterine epithelium during diestrus are attributable to cellular degeneration. The small droplets appear to indicate cellular activity, whereas the large droplets are associated with cellular inactivity (20).

Recently, Nicol and Snell (21) reported briefly on the hormonal control of lipide in the reproductive tract of the female guinea pig. They concluded that in the uterine epithelium the lipide appears to be an index of cell activity but in the vaginal epithelium it is the result of a process of fatty degeneration. They described also large lipide-containing, reticulo-endothelial cells in the stratum compactum, which are most numerous about the eleventh day. There are reasons to question their identification of these lipide-containing cells as elements of the reticulo-endothelial system. It was possible in the present study to follow the appearance of very intensely sudanophilic cells in the uterine and the vaginal connective tissues. However, they apparently do not contain true lipides but give false sudanophilic reaction due to a substance insoluble in acetone. These cells, which are of the same size as leukocytes, are tentatively identified as neutrophiles and tissue eosinophiles. Staining of eosinophilic and neutrophilic leukocyte granules by sudan black B has been reported by McManus (22), Discombe (23), Wislocki and Dempsey (24) and others, and was interpreted as due to the presence of lipides in the granules. However, it has recently been demonstrated by Lillie and Burtner (25) that the sudanophilia of these granules is not due to differential solubility of the sudan dye as in the case of the staining of true lipides, but is the result of an unexplained, chemical combination of the dye with some other cytoplasmic constituent.

We agree with Selle and Tribby in the division of the cycle of the female guinea pig into four stages, and like them we were unable to detect any evidence of vaginal hemorrhage as described by Stockard and Papanicolaou (1). During estrus and metestrus, the free border of the surface and glandular epithelia of the entire endometrium appears to be striated. This finding is in disagreement with the description of Stockard and Papanicolaou of a typical ciliated epithelium. The same authors described also an intense secretion of mucus by the endometrial surface epithelium during estrus, with many of the cells filled with mucus, but we were unable to confirm this finding. In our preparations which are longitudinally cut, it is apparent that mucous cells are present solely in the cervix and stop

abruptly in the juxta-cervical region of the endometrium. Moreover, these cervical mucous cells are present throughout the cycle.

In our preparations, it is apparent that the uterine surface epithelium remains completely intact during the entire cycle and that terminal bars are present at all times in both the surface and glandular epithelia. These findings are at variance with the earlier description of Stockard and Papanicolaou who reported the shedding of the entire surface epithelium during diestrus. It is possible, however, that the shedding described by them occurred during part of the interval of mid-diestrus that we did not study. Moreover, uterine hemorrhage and hematomata such as they noted were absent in all of our specimens.

The cervical epithelium presents two clearly distinct layers of cells throughout the cycle: a basal layer of cuboidal cells and an outer layer of mucous columnar cells. A similar condition is reported in the uterine cervix of the rat by Hamilton (26). She describes two regions: a rostral, uncornified one and a caudal one essentially similar to the vagina. We have observed three zones: first, a two-layered central region with deep, branched, tubular glands composed of basal and mucous cells; second, a juxta-uterine region with short wide invaginations consisting of a basal layer continuous with the uterine surface epithelium and a mucous layer which stops abruptly; and, third, a juxta-vaginal region of stratified basal epithelium which is cornified during late estrus and an outer mucous layer which becomes continuous with the vaginal mucous cells during late diestrus and proestrus.

That the "sexual skin" undergoes periodic development and regression correlated with ovarian function during the menstrual cycle has been reported in monkeys by numerous authors (27; 28; 29; 30; 31; 32; and others), but we are unable to find in the literature any reference on the "sexual skin" of the guinea pig. Changes which extend from a thick and highly cornified epithelium with a well-developed stratum granulosum during estrus, to a lower and poorly cornified epithelium without a stratum granulosum during diestrus, indicate that the skin of the guinea pig's genital orifice also undergoes periodic changes during the cycle which justify its being regarded as "sexual skin." A curious fact reported here is the behavior of the melanin-containing cells of the sexual skin during the cycle. In our animals, melanin was present throughout the cytoplasm of the entire epithelium during late proestrus and estrus but completely absent from the epithelium and confined to the stroma during metestrus and diestrus. The small number of animals studied with respect to this phenomenon do not permit us to draw definite conclusions, but one of us has observed a somewhat similar phenomenon in the thumb pad of Rana pipiens (33). Melanophores appeared between the epithelial cells of the epidermis, predominantly during the breeding season and under gonadotrophin stimu-

lation. These observations relating to melanin deserve further investigation.

A comparison of the guinea pig's sexual skin and vaginal epithelium reveals striking differences in morphology and histochemistry. A narrow transitional zone is recognizable between the two epithelia. Keratohyaline granules present in the skin are absent in the vaginal epithelium, and a disulfide reaction which occurs only in the cornified layer of the skin is distributed throughout the entire width of the vaginal epithelium. Alkaline phosphatase, glucose-6-phosphatase and aliesterase are all present in the vaginal epithelium but are not demonstrable in the sexual skin. These findings point to different processes of metabolic activity in the skin and vaginal mucosa and to the possibility that the mechanism of cornification of the two structures may be different. It is noteworthy that keratin formation has never been conclusively demonstrated in the vaginal epithelium.

In closing, we wish to recall that the histochemical observations made in this study will be correlated in a subsequent paper on results recently obtained with the electron microscope. For example, electron microscopy has revealed the unexpected presence of microvilli on the free borders of endometrial surface cells and glandular epithelium, present only during estrus and metestrus. Similarly, microvilli develop on the plasma membrane of the basal and prickle cells of the vagina during the same periods of the cycle.

SUMMARY

This paper describes the cyclical changes in the uterus, cervix, vagina and sexual skin of the guinea pig investigated by histological and histochemical methods. The only previous study of any of these structures by histochemical means was confined to the vagina and was limited to observations on glycogen, mucus, and lipides. The present study concerns an investigation of all of the structures enumerated above with reference to the presence and distribution of cytoplasmic basophilia, mucus, glycogen, lipides, protein-bound disulfide groups, alkaline glycerophosphatase, glucose-6-phosphatase and aliesterase.

The detailed changes in the amounts and distribution of the various substances in the epithelia of the uterus, cervix and vagina, investigated with respect to the phases of the reproductive cycle, are tabulated.

In disagreement with the accounts of some previous investigators, the surface and glandular epithelia of the uterus appear to possess a transitory striated border instead of cilia, and the surface epithelium contains no mucous cells. Terminal bars are associated with the surface and glandular cells throughout the cycle and the surface epithelium appears to remain completely intact at all times. No uterine or vaginal hemorrhages were observed. Cytoplasmic basophilia, present chiefly in the surface epithelium, is attributable to ribonucleoprotein. Glycogen is limited to traces in the

surface epithelium during estrus and to the endometrial stromal cells during metestrus.

The cytology of the juxta-endometrial and juxta-vaginal regions of the cervix is described with respect to the phases of the cycle. The mucous cells of the cervical epithelium display the histochemical reactions of an acid mucopolysaccharide. The maximal accumulation of mucus in the cervical epithelium occurs during estrus, whereas the uterine epithelium develops maximal secretory activity in metestrus.

The vagina displays a number of interesting reactions. The elaboration of mucus starts near the vaginal orifice in late diestrus and spreads rostrally to reach the upper end of the vagina during proestrus. Cornification begins at the vaginal orifice in late proestrus and spreads rostrally to reach the cervix at the end of estrus, as observed by Stockard and Papanicolaou. Glycogen appears in moderate amounts in the vaginal epithelium during proestrus and is correlated with the appearance and distribution of glucose-6-phosphatase.

The guinea pig possesses a "sexual skin" in the sense that the epidermis of the genital outlet undergoes cytological and histochemical changes during the reproductive cycle, differing from those of the vagina. Furthermore, comparisons of the histochemical reactions of the vaginal epithelium and the epidermis of the sexual skin suggest that the two structures may possess different metabolic activities and mechanisms of cornification.

Small lipide droplets which characterize the vaginal, cervical and uterine epithelia during proestrus, estrus and metestrus, suggest cellular activity, whereas large lipide droplets in the vaginal desquamating layers during proestrus and estrus, and in the cervical and endometrial epithelia during diestrus, indicate cellular regression.

The present histological and histochemical observation constitute background material for a study, nearing completion, on the electron microscopy of the same region of the guinea pig's reproductive tract.

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GROWTH AND DEVELOPMENT OF TOOTH GERM HOMOTRANSPLANTS IN FEMALE GUINEA PIG CASTRATES[1]

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INTRODUCTION

IT HAS been observed (7) that during growth, differentiation and development of tooth germ transplants, associated cells of such explants undergo progressive specialization and functional activity so that following mineralization, calcified components of the teeth are ultimately formed. Continuation of these studies (8, 9, 10, 11, 12, 13) has led to transplanting the anlagen of teeth under various environmental conditions with the objective of altering their subsequent growth and development. This communication is concerned with the effect of ovariectomy on the growth and development of tooth germ homotransplants in guinea pigs.

Gardner (15) reported that large amounts of estrogens resulted in stunting the body growth of mice. Beebe (3) and more recent investigators (4, 16, 20) have reported inconsistent findings with body weight and increase in size of various organs in studies with female castrates. It is generally accepted, however, that when estrogens are present, body growth is inhibited and castration of the female induces greater body growth than would ordinarily occur (16). It has also been established that tooth germ transplants become a functional part of the hosts through connective tissue attachments and vascularization (13). Thus, such transplants as functional parts of castrates should have alterations in growth and development. There have been no reports on the biological activity of embryonic tissue transplants such as tooth germs in ovariectomized animals.

MATERIALS AND METHODS

Young adult female guinea pigs weighing approximately 200–400 gm. were anesthetized with intramuscular injections of fresh veterinary nembutal. Animals were first shaved in the costo-vertebral areas and then the ovaries were exposed and excised. Uterine horns were tied off with black silk before closing the incisions. From five to ten days were allowed for recovery before transplants were made to the eyes and brains of these animals.

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The first and third cheek teeth anlagen of 25–35 day guinea pig embryos were considered most satisfactory for transplantation for several reasons. First, because in embryos of the above age they were still uncalcified, and second, because sufficient numbers could be obtained from a single group of fetal mates for a representative series of transfers. Older embryos, up to 45 days of age, were not satisfactory because only the 4th cheek anlagen could be utilized. Anlagen of the 2nd cheek teeth were sometimes used in these experiments.

The techniques of transplantation of tooth germs to the eyes and brains of experimental animals have previously been described (7, 8).

In each series from 8 to 16 ovariectomized guinea pigs received homotransplants of tooth germs from a single group of fetal mates with additional transplants, to serve as controls, being made to from 4 to 6 intact females. In a number of series approximately half the ovariectomized hosts bearing transfers were given intramuscular injections of 1 mg. of diethylstilbestrol 3 to 5 times a week for four weeks. This replacement therapy was given as a substitute for natural gonadal secretions so that growth of transplants could be compared with those in intact females and untreated ovariectomized animals.

Animals were sacrificed intermittently from 12 to 200 days and transplanted tissues recovered. When possible, moribund hosts were sacrificed at once and the transplants recovered. On death or sacrifice, all recovered transplants were fixed, decalcified, sectioned and stained with haemotoxylin and eosin. Autopsies were routinely performed on all sacrificed hosts to discover if any vestiges of ovarian tissues remained. Uteri were always examined to determine the extent of atrophy and degree of vascularity. Adrenals and pituitaries were examined and representative specimens sectioned and stained for comparative histologic study.

In Table 1 the statistics of these studies with ovariectomized animals are listed. The

TABLE 1. STATISTICS OF OVARIECTOMIZED STUDIES

Total No. of Ovariectomized Animals Bearing Transplants			231
Total No. of Untreated Ovariectomized Animals with Transplants			137
Total No. Takes in Untreated Ovariectomized Animals			103
Total No. Diethylstilbestrol Treated Animals with Transplants			94
Total No. of Takes in Diethylstilbestrol Treated Animals			63
Total No. Transplants Made to Intact Animals			90
Total No. Takes in Intact Animals			74
	Group 1. Gross and Microscopic Appearance in Untreated Hosts		
Takes	+.+++52		
		Animals Lost or Died and No Transplants Recovered	21
	+++25		
	++16		
		Transplants Infected and/or Resorbed	13
	+10		
	Group 2. Gross and Microscopic Appearance in Diethylstilbestrol Hosts		
Takes	++++10		
		Animals Lost or Died and No Transplants Recovered	19
	+++29		
	++15		
		Transplants Infected and/or Resorbed	12
	+ 9		
	Group 3. Gross and Microscopic Appearance in Intact Hosts		
Takes	++++ 9		
		Animals Lost or Died and No Transplants Recovered	11
	+++27		
	++26		
		Transplants Infected and/or Resorbed	5
	+12		

TABLE 2. TRANSPLANTATION EXPERIMENT WITH OVARIECTOMIZED GUINEA PIGS

Quality of Take	Group 1	Group 2	Group 3	Group 4
		Observed Values		
++++	52	10	9	71
+++	25	29	27	81
++	16	15	26	57
+	10	9	12	31
−	34	31	16	81
Total	137	94	90	321
		Expected Values		
++++	30.30	20.79	19.91	71
+++	34.57	23.72	22.71	81
++	24.33	16.69	15.98	57
+	13.23	9.08	8.69	31
−	34.57	23.72	22.71	81
Total	137.00	94.00	90.00	321
		O—E		
	22	−11	−10	
	−10	5	4	
	− 8	− 2	10	
	− 3	0	3	
	− 1	7	− 7	
	0	− 1	0	

$P < 0.001$.

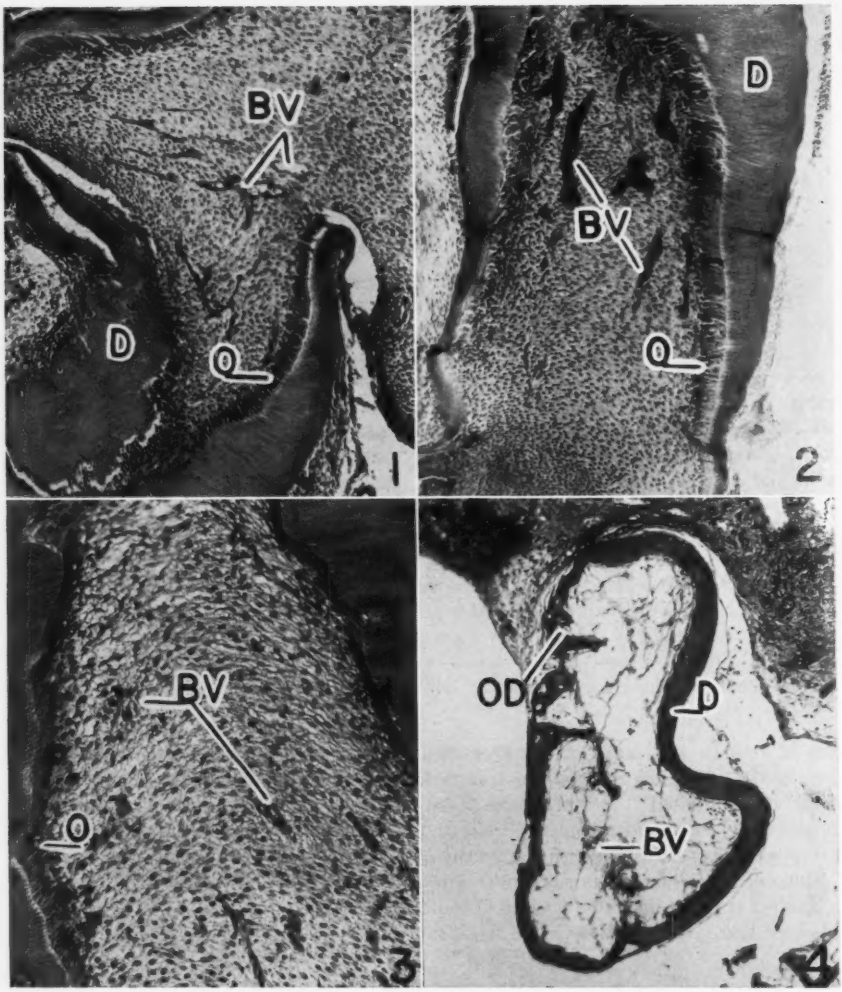
eyes of animals with anterior chamber transplants were examined periodically to determine the biological behavior of transfers. Growth in animals bearing brain transplants was evaluated at autopsy. Both these groups are listed together with the quality and size of recovered transplants indicated as follows: ++++ = excellent growth; +++ = good growth; ++ = fair growth; + = poor growth; and − = no growth.

Reasons for failure of transplants to grow are included in Table 1.

Table 2 is based on Table 1 and it is indicated by this latter table that the observed values of the quality of takes were significantly greater than the expected values.

RESULTS

For the period of these experiments, tooth germ transplants in ovariectomized animals matured or aged at a slower rate than in intact hosts. Thus, epithelial and mesenchymal cells associated with these embryonal transplants continued their biological activity for longer periods than was usually found in intact animals. In the ovariectomized hosts, growth was considerably augmented, for more extensive amounts of pre-enamel and pre-dentin were laid down (cf. Fig. 3 and 4); and frequently by 55 to 65 days, the corneas were pierced by the cusps of these developing transplants. Often the cusps curved as they grew, adapting themselves to the confines of the areas to which they were transplanted. This was observed with both brain and anterior chamber transplants (Fig. 9 and 11). By actual weight and size, transplants frequently grew larger than comparable cheek teeth in



EXPLANATION OF FIGURES

FIG. 1. To show pulpal vessels and cells in anterior chamber transplant in untreated ovariectomized guinea pig after 30 days. Donor tissue, 3rd cheek tooth bud from lower jaw, 28 day guinea pig embryo. Original magnification ×130.

FIG. 2. To show pulpal vessels and cells in anterior chamber transplant in diethylstilbestrol treated ovariectomized guinea pig after 30 days. Host animal received 12 intramuscular 1 mg. injections of diethylstilbestrol. Donor tissue from same group of litter mates as Figure 1 and also the same type of tooth germ. Original magnification ×130.

FIG. 3. To show pulpal vessels and cells in transplant to anterior chamber of untreated ovariectomized guinea pig after 60 days. Donor tissue 3rd cheek tooth upper jaw from 30 day guinea pig embryo. Note cellularity of pulpal area and odontoblasts along pulpal periphery. Original magnification ×220.

FIG. 4. To show avascularity and acellularity of transplant in anterior chamber after 57 days in intact female guinea pig. This transplant from same series of fetal litter mates as in Figure 3. Tooth germ also the same. Original magnification ×145.

the hosts' own jaws. Growth and development of transplants in diethylstilbestrol treated animals was usually greater than in intact control hosts but these seldom attained the size of transplants in untreated ovariectomized hosts. There was more supporting tissue about transplants in intact animals than in either group of ovariectomized animals. Transplants that looked very large in the anterior chambers of intact hosts were found on microscopic examination to have more abundant connective tissue in relation to the transplants than in the castrated hosts. It was also observed that transplants matured earliest in intact control hosts, latest in untreated castrates, while those in diethylstilbestrol treated castrates were intermediate.

Gross and histologic examination of eyes bearing transplants showed that when a peak of vascularity was reached in transplants in ovariectomized animals, it was maintained for longer periods than in intact hosts. Pulpal areas in transplants in untreated and diethylstilbestrol treated ovariectomized animals maintained a greater number of blood vessels than was found in intact hosts. In the diethylstilbestrol treated hosts, vessels in transplants were large and more heavily engorged with red blood cells (Fig. 2) than in the untreated ovariectomized animals. At times, this engorgement was to the point of stasis and often small hemorrhages were found in the pulps.

Pulpal areas of transplants in untreated and treated ovariectomized animals retained a much greater degree of cellularity than did those in intact animals (Figs. 1–3). In the first mentioned instances, pulps were filled with closely packed cells among which mitotic figures were numerous. When cellularity ultimately decreased and before osteoid changes took place (Fleming, 11), numbers of plasma cells and eosinophils appeared within the pulpal areas. In addition to the above, odontoblasts did not readily disappear from the pulp, but remained closely packed in their normal positions. Their nuclei were large and dark staining. In places where odontoblasts had disappeared, osteoid revision of the pulpal areas had begun. In some instances, these changes were quite extensive as compared with those usually found in transplants in intact animals. Although young osteoid tissue, in such instances, was predominantly present, tissue of a cartilagenous-like appearance was likewise frequently found. These cartilage-like structures had large cells that took a decidedly pink stain (Figs. 5 and 6).

Ameloblasts in the transplants in castrated animals were also closely packed together and appeared to remain active for longer periods than those in intact animals. While dentin formed in these transplants presented the regular appearance of tubular dentin, the enamel did not always present

Key to Figures: A = ameloblasts. E = enamel. O = odontoblasts. D = dentine. C = cartilage. BV = blood vessels. OD = osteodentine. OS = osteoblasts. PC = plasma cells.

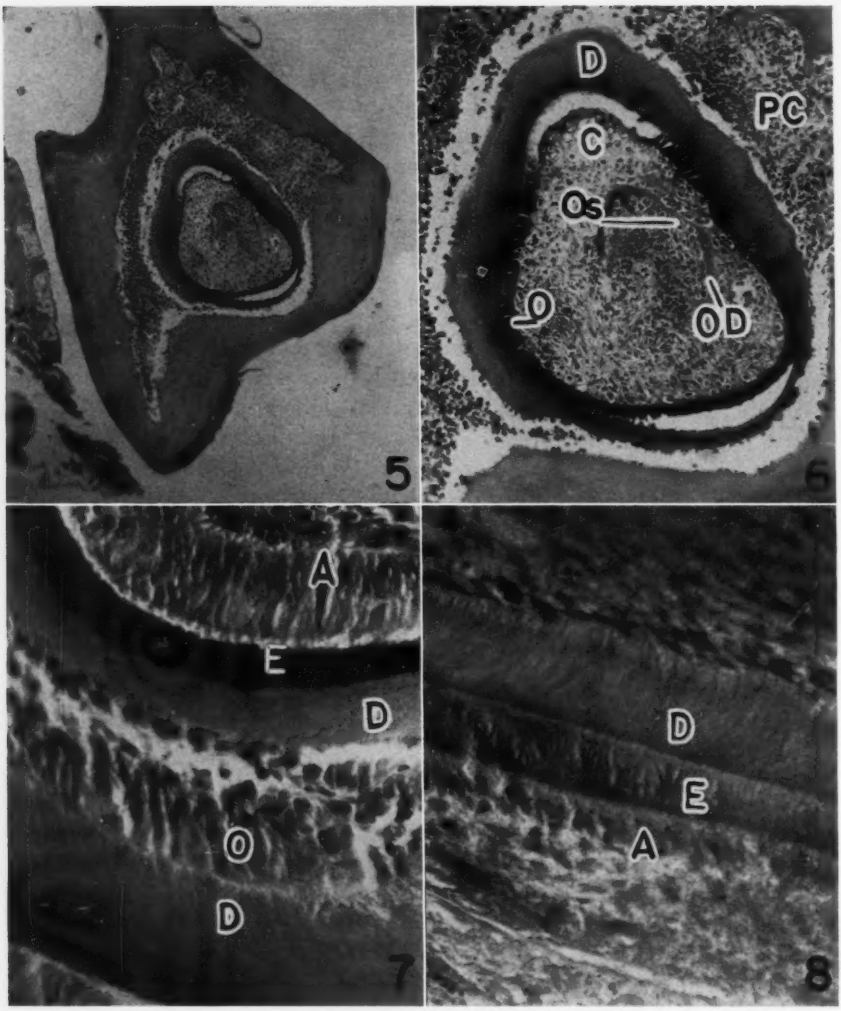
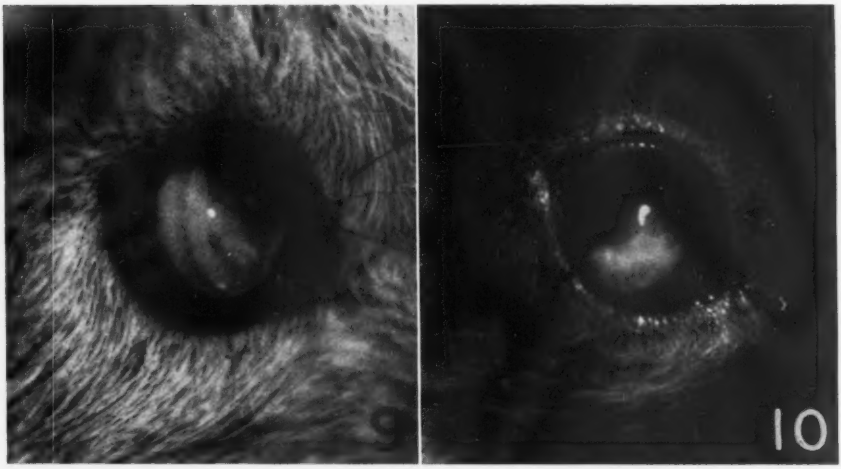


FIG. 5. Anterior chamber transplant after 85 days in untreated ovariectomized guinea pig. Donor tissue 1st cheek tooth of 27 day guinea pig embryo. Original magnification ×75.

FIG. 6. Higher magnification of Figure 5 to show revision of pulpal area. In upper center of pupal area there are cartilage cells; at right center, osteoblasts and osteoid tissue. Most cells along outside of dentine are plasma cells and eosionphils. Original magnification ×200.

FIG. 7. Higher power of an area not seen in Figure 1 to show condition of enamel matrix (upper and lower dark staining bands) also the numbers of ameloblasts. Original magnification ×550.

FIG. 8. Higher power of an area in Figure 2 to show difference of enamel formation in transplants in a diethylstilbestrol treated host. Original magnification ×550.



FIGS. 9 and 10. Transplants after 65 days in anterior chamber of guinea pigs in Figure 9, host has been ovariectomized while in Figure 10 the host is intact. Tooth germs were obtained from 31 day old guinea pig embryonic litter mates. Both tooth germs were 1st cheek teeth.

the expected appearance on the aspect bordering toward the Tomes' processes; the ends of the enamel rods were greatly twisted (Fig. 7). This phenomenon has not been previously seen in these transplantation experiments.

DISCUSSION

The augmented growth and development of a significant number of tooth germ transplants in untreated ovariectomized guinea pigs was the major observation in the present series of experiments. Transplants in diethylstilbestrol treated castrates as well as those in intact hosts failed to attain this superior growth. Previous studies with tooth germ transplants have shown that following a period of growth and development, mineralized tissues matured and calcified (Fleming, 7). There followed an osteoid revision of the pulpal areas of these transplants as they gradually became avascular and acellular (Fleming, 11, 13). Ultimately, this revision, which was at times evident as early as 30 days following transplantation, involved the calcified dentine of these transplants. In the present series of experiments with ovariectomized animals, transplants continued growth for much longer periods, and pulpal changes did not occur at such an early date.

Growth hormonal activity results in a synthesis of tissue proteins from amino-acids and carbohydrates; fats and minerals play a part in this synthesis (Gaunt, 16). Thus, other trophic hormones of the anterior pituitary, either directly or indirectly, can have rate limiting effects on growth. It is

therefore difficult to evaluate the exact roles of these end-organ metabolic responses which are mediated by the various pituitary trophins; all of which help regulate the enormously complex biological phenomenon of growth.

Very few investigations dealing with mineralized tissues in ovarectomized animals have been concerned with the teeth. Two reports dealing with caries susceptibility in ovariectomized animals did not agree. Granados *et al.* (17) reported that ovariectomy increased caries suceptibility in hamsters. Delman (6) in a non-conclusive report stated that there is no statistical reduction of caries in ovariectomized rats.

Pinborg (19) in his studies found no changes in bones nor in calcification

FIG. 11. Shows comparative size of transplants. The middle tooth was in the brain of an ovarectomized guinea pig for 85 days. Bottom tooth is the comparable tooth from the host's mouth, a 3rd upper cheek tooth, in a 700 gram guinea pig. Top tooth was removed from brain of a female castrate after 85 days that had received 15 intramuscular diethylstilbestrol injections during the first four weeks of transplantation. Tooth germs were obtained from 28 day guinea pig embryos.

of teeth in female rat castrates. In comparison with the present experiments, these observations are not consistent, for growth and development of tooth germ transplants was prolonged in castrated female guinea pigs and there was an accompanying retardation of calcification. Bauer (2), while dealing with the effect of estrogens in young puppies, observed changes in the teeth and jaws. This study was similar to the previous work of Gardner and Pfeiffer (15) who were concerned with the effect of estrogens on the skeletal systems of birds, dogs, and guinea pigs. Bauer found that the snouts of treated puppies were somewhat shorter and in these instances there was an increased density of bone between the erupting buds of the permanent teeth. There were no changes in enamel and dentin but there were differences in the outer enamel epithelium layer of cells and the stratum intermedium. In addition, as was observed in the present stud-

ies when diethylstilbestrol was administered, there was an increase and congestion of capillaries within the dental sac.

One possible explanation of the increased growth of transplants, in the present experiments, is that there was an alteration of the host-transplant relationship in the castrates. It has been reported by Harris and Eakin (18) that following ovariectomy the increase of circulating gonadotrophins diminished antagonistic responses by the hosts to alien cells. Thus, transplants which are to assume a functional status in their ovariectomized hosts could presumably be stimulated to a higher degree of metabolic activity by a lowered incidence of antagonistic responses. Therefore, a difference in host-transplant relationship could minimize antibody responses. This could explain the fact that supporting tissue about transplants in untreated ovariectomized animals was less than that in intact hosts or in treated castrates. Connective tissue about the eye transplants in normal hosts often gave these transplants the appearance of being considerably larger than they actually were. Presumably the smaller amount of connective tissue about transplants in ovariectomized animals was not of sufficient quantity to restrict their growth. The cellular elements of the blood assumed a different pattern about transplants in ovariectomized animals for lymphocytes did not appear in large groups or in definite focal concentrations about such transplants. However, in transfers of long-standing, there was a greater number of plasma cells associated with transplants than was usually found.

Another possible cause for the augmented growth could have been the persistence of an active blood supply. It has been previously observed by Gardner (14) that estrogens elicit hyperemia of certain body tissues. As a result, alteration of the vascular supply of myeloid and endosteal tissues may favor osteoblastic proliferation and calcification. In the present studies the elaboration of mineralized tissues was not entirely dependent upon the quantity of blood supply. This was indicated by the fact that in diethylstilbestrol treated animals, transplants had the greatest blood supply, yet these transplants never attained the size of those in untreated ovariectomized hosts.

There are several reasons why growth of transplants was not consistently of the same high quality in the female castrates. Condition of the tissues to be transplanted and condition of the hosts as well as the transplantation site has been considered previously (Fleming, 7 and 8). In addition, in the present study, there could have been an endogenous source of estrogen following ovariectomy which when present might have exerted rate-limiting effects on growth and development. This has been noted by Aron *et al.* (1) in a study with ovariectomized guinea pigs where it was observed that in 6 out of 39 animals varying degrees of an endogenous source of estrogen existed. Cases *et al.* (5) also observed that when ovariectomized mice received full diets, there were evidences of estrogen stimulation in the uteri

and this was in contradistinction to those animals on restricted diets. In view of these observations, an explanation may be offered concerning variations noted in growth of transplants as indicated in the accompanying tables.

SUMMARY

Augmented growth and development of a significant number of homotransplanted tooth germs was observed in ovariectomized guinea pigs. There was a lowered incidence of antagonistic responses by the hosts to transfers, the persistence of an active blood supply and a failure of ameloblasts and odontoblasts as well as associated cells to age as they did in intact hosts. When diethylstilbestrol was used, transplants failed to attain the size of those in untreated castrates, but they were larger than those in intact animals. Transplants matured earliest in normal hosts, next in diethylstilbestrol treated castrates, and last in untreated castrates. Possible explanations of these phenomena are included.

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NOTES AND COMMENTS

LACK OF BIOLOGICAL ACTIVITIES OF 21-HYDROXYPREGNANE-3,20-DIONE SODIUM SUCCINATE[1]

The anesthetic properties of 21-hydroxypregnane-3,20-dione sodium succinate have recently been reported (1, 2, 3). In view of the structural relationship of hydroxydione with many hormonally active steroids, the need for investigating the hormonal activity of this steroidal anesthetic was apparent. In all experiments reported herein, hydroxydione was administered in aqueous solution. The free acid of hydroxydione was obtained and made water soluble for injection by neutralizing with an equivalent amount of sodium carbonate. The dosages given throughout this communication represent those of the free acid. Organ weights were taken on a Roller-Smith Torsion balance to an accuracy of 0.01 mg.

We have assayed crystalline hydroxydione for estrogenic, androgenic, anabolic, progestational, glucocorticoid and adrenalcorticotrophic activity with negative results (Table 1).

The electrolytes and water excretion following injection of hydroxydione were carefully studied according to the method of Cook *et al.* (10) in bilaterally adrenalectomized rats. Comparison studies were also made with desoxycorticosterone acetate and thiopental sodium. The results are presented in Table 2. It will be seen from these results that subcutaneous injection of hydroxydione or thiopental sodium in adrenalectomized rats resulted in a decrease in sodium and water excretion. However, the influence of hydroxydione (or thiopental sodium) and desoxycorticosterone on electrolytic and water metabolism can easily be distinguished. The retention of sodium and water by desoxycorticosterone is accompanied by an increase of potassium excretion while the reduction of sodium output by hydroxydione or thiopental sodium is usually accompanied by a reduction of potassium excretion. The decrease in the excretion of urinary

TABLE 1. LACK OF BIOLOGICAL ACTIVITY OF HYDROXYDIONE

Activity tested	Assay method	Animal species	No. of animals	Dosage range mg.	Result
Estrogenic	Astwood (4)	Rats	180	.01–5.00/rat	Negative
Androgenic and Anabolic	Herschberger (5)	Rats	173	3.5–50/rat	Negative
Progestational	Corner and Allen (6)	Rabbits	16	50–525/kg.	Negative
Corticoid					
Glycogenic	Venning (7)	Mice	50	1–5 /mouse	Negative
Thymolytic	Dorfman (8)	Mice	92	0.1–5 /mouse	Negative
Adrenalcortico-trophic	Sayers & Sayers (9)	Rats	31	30 /kg.	Negative

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[1] Viadril brand of hydroxydione is the trade name applied to 21-hydroxypregnane-3,20-dione sodium succinate by Chas. Pfizer & Co., Inc.

TABLE 2. THE EFFECT OF HYDROXYDIONE AND SODIUM PENTOTHAL ON WATER, SODIUM AND POTASSIUM EXCRETION IN ADRENALECTOMIZED RATS

Treatment	Dosage	Mortality ratio*	Urine volume	Sodium	Potassium
	γ/rat		cc./6 hr.	mg./hr./100 gm.	mg./hr./100 gm.
Control	0	0/20	6.8 ±0.22†	5.04±0.23†	5.78±0.28†
DCA	15	0/20	4.9 ±0.18	3.29±0.31	6.03±0.27
DCA	50	0/20	5.1 ±0.27	3.32±0.69	6.48±0.43
Viadril	1,000	2/20	5.1 ±0.39	3.27±0.45	4.99±0.59
Viadril	5,000	4/20	2.9 ±0.35	1.88±0.45	2.96±0.21
Viadril	10,000	9/20	1.2 ±0.56	0.86±0.56	1.83±0.21
Pentothal Sodium	1,000	5/20	3.7 ±0.91	2.85±0.45	3.57±0.44
Pentothal Sodium	5,000	2/20	2.3 ±0.76	1.72±0.70	2.05±0.39
Pentothal Sodium	10,000	15/20	1.35	0.81	1.42

* No dead/No. treated.
† Standard Error.

sodium and potassium observed in rats which received hydroxydione or thiopental sodium is most likely due to a decrease in renal activity during anesthesia. Merrill (11) has pointed out that deep anesthesia can influence the renal handling of sodium and thus result in sodium retention. Silvette (12) has shown that injection of anesthetic doses of thiopental sodium reduces or prevents water diuresis during the duration of anesthesia. Habif, *et al.* (13) also showed that the barbiturates were like ether and cyclopropane in inducing antidiuresis. The mechanism by which barbiturates decrease the excretion of urine has been a subject of controversy. Silvette (12) reported that thiopental sodium produces antidiuresis in both intact and hypophysectomized rats. He attributed the cause of pentothal sodium antidiuresis to either renal or general hypotension and not to a direct affect on the kidney or on the hypothalamicohyophyseal system. On the other hand, De Bodo and Prescott (14) have shown that barbiturates induce antidiuresis through the release of antidiuretic hormone. The authors felt that Silvette's work did not contradict their finding but they were rather in full agreement. They pointed out that in the hypophysectomized animal without permanent polyurea both morphine and barbiturates will prevent water diuresis. However, in neurohypophysectomized animals with permanent diabetes insipidus neither of these drugs inhibits water diuresis. De Bodo and Prescott (14) felt that Silvette's studies fall into the former class and not the latter.

It is quite probable that hydroxydione, like other anesthetics, may also affect urine and sodium excretion through a release of the antidiuretic hormone. Further studies are in progress to elucidate the mechanism of action of hydroxydione antidiuresis.

SUMMARY AND DISCUSSION

Hydroxydione has been bioassayed for estrogenic, androgenic, anabolic, progestational, glucocorticoidal and adrenalcorticotrophic activity and was found to be inactive. Although hydroxydione causes retention of sodium and water in adrenalectomized animals, it differs from the actions of desoxycorticosterone in that the retention of sodium by desoxycorticosterone is accompanied by an increase of potassium output whereas the reduction of sodium excretion by hydroxydione is usually accompanied by a reduction of potassium excretion.

Since hydroxydione was given in aqueous solution, its rapid renal clearance may not enable one to evaluate the potential endocrine activities of this compound. However,

the data presented here are at least adequate to indicate that patients receiving an anesthetic dose of hydroxydione are not likely to manifest any significant endocrine side effects.

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ALLOXAN ADMINISTRATION IN THE GUINEA PIG WITH ACINOUS TISSUE ATROPHIC RENDERED BY LIGATURE OF THE PANCREATIC DUCT

The conversion of acinous and centroacinous pancreatic cells into islet cells has been for a long time the subject for discussion. There exists evidence that acinous tissue has a potential capacity to convert itself into insular tissue, which furthermore is capable, under emergency conditions to build additional islets (1, 2, 4, 7, 9). Five to six weeks following ligation of pancreatic duct proximal to papilla Vateri (Allegretti and Dolinar, unpublished), the acinous tissue atrophies, the common pancreatic duct and its branches are enlarged and acini are presented as small tubes surrounded by cells, which are more or less flattened. It is presumed that the atrophy is brought about by increased pressure within the closed system of pancreatic ducts. Similar tube-shaped formation may also

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be seen in several islets. Bensley has shown that islets of the guinea pig, and other animal species, are connected with a system of small tubes, which are joined with the large ducts. As a cause of these tube-shaped formations in the islets it is possible to suppose that a) the small tubes are connected with the large ducts, and the increased pressure produced by the ligature causes their extension; b) the cells surrounding these formations and the time of ligation were just developing from the small tubes, or ducts thus making the islets, and the increased pressure produced the tube-shaped formations; c) these surrounding cells develop from acinous cells so that the final appearance is very similar to that of the acinous remnants following ligation. The cells of the tube-shaped formations in the islets show no difference when compared with other islet cells, and there exists no proof that they originate from the small tubes or the ducts. The cells of the normal islets of the guinea pig are often arranged after the pattern or the acinous cells.

All the above findings speak in favour of the presumption that islets might also originate from the acini, although Bensley does not agree with this concept. He could not find any transition forms from the acinous to the islet cell.

Johnson (5, 6) described transition forms of acinous to β-cells in the guinea pigs previously injected with alloxan. The presumption that the tissue has ability to convert itself into the islet tissue, as the above mentioned typical arrangement of islet cells, corroborate her findings. She concluded that the guinea pig is resistant to the diabetic action of alloxan on account of the great ability of the islets to regenerate or to develop from the acinous tissue.

In the guinea pigs with the atrophied acinous tissue following the ligation of the main pancreatic duct the injection of the alloxan should produce diabetes, if Johnson's hypothesis is correct.

For this purpose the main pancreatic duct was ligated in six male and five female guinea pigs. They were of 206 to 481 gm. body weight. Six weeks after operation alloxan was injected in amount of 100 mg. per kg. of body weight into jugular vein using ether narcosis. Blood sugar level was determined after the Hagedorn and Jensen method. The determination of blood sugar was performed before the injection, then (if possible to obtain the blood sample from the ear wein) 10, 20, 30 minutes, 1, 2, 3, 4, 5, 8, 12, 24, 48, 120 hours and 7 and 15 days after the injection of the drug. Four male and three female animals lived for fifteen days.

By analyzing the glycemic curve (Fig. 1.) the first transient hyperglycemic phase is clearly visible, which returns to normal level during a period of two hours. According to Fig. 1. it is not possible to deny the existence of the transient hypoglycemic phase by reason of the small number of the blood sugar determinations during the corresponding period. Five animals died during the period of experiment. One animal died ten minutes, the second 20, and the third 30 minutes, following the injection, i.e. the death took place in the first transient hyperglycemic phase. Death was caused by narcosis and not by hypoglycemia. Two animals died 24 and 48 hours respectively after injection, when hypoglycemia has already disappeared. The animals were controlled for 24 hours after injection and no signs of convulsions were detected, so that glucose application was unnecessary. By further inspection of the glycemia curve a slight hyperglycemia may be observed 24 and 48 hours after injection, which returns to normal value on the fifth day.

Fifteen days after the injection no animal had a blood sugar level higher than 160 mg. /100 ml. hence we presume that none was diabetic. On the fifteenth day after injection the animals were not fasting, which is the cause of somewhat higher blood sugar values then is the fasting level. By histological examinations of tissue slides stained by Gomori's method it could be observed that acinous tissue practically disappeared and normal islets were all about in the dense bundle of the connective tissue.

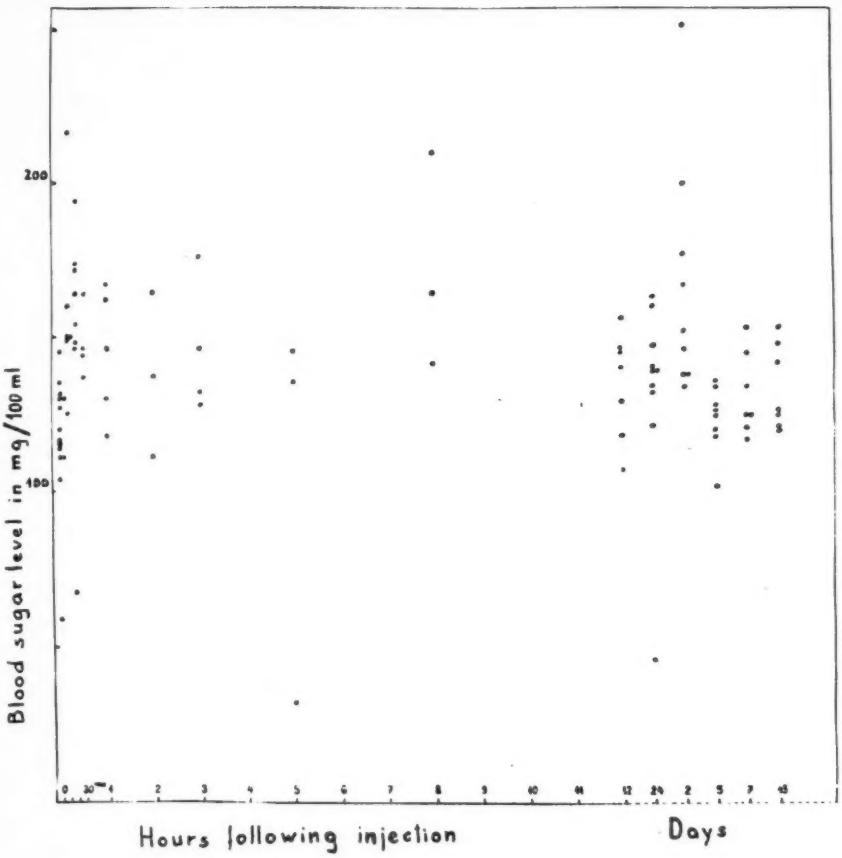

FIG. 1

By the ligature of the main pancreatic duct the acinous tissue atrophied during five to six weeks thus producing the disappearance of that tissue from which according to Johnson, originate new β-cells in a few days. The inability of alloxan to produce diabetes in this way could be explained in two manners: a) either contrary to Johnson's claims the acinous tissue is not able to produce new islets, at least not in a few days or b) the islets of the guinea pigs are in themselves resistant to the diabetic action of alloxan. All in all the resistance of the guinea pigs to the alloxan may not be explained by the islets regenerating from the acinous cells.

Objection might be raised to the above conclusions on the basis of the results of Walpole and Innes (8). They produced in rabbits a resistance to the diabetic action of alloxan if the acinous tissue had been rendered atrophic by means of ligature of the duct. Similar experiments on dogs carried out by Goldner and Gomori (3) do not corroborate these results. This will be the subject of further investigations.

SUMMARY

The main pancreatic duct in guinea pigs was ligated, and six weeks afterwards a diabetic dose of alloxan was injected. The acinous tissue became completely atrophic. Diabetes did not appear, which is contrary to the presumption, that guinea pigs are insensitive to alloxan on account of the ability of the acini to form new islets in a short time.

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RADIOIODINE NOT DISCHARGEABLE WITH PERCHLORATE IN THE THYROIDS OF PROPYLTHIOURACIL TREATED RATS[1]

Thiocyanate (1, 2) and perchlorate (3) interfere with the active uptake ("trapping") of iodide ions by the thyroid and discharge concentrated iodide which is present in the gland at the time of their administration (2, 3). In rats, complete inhibition of thyroidal iodide "trapping" apparently cannot be achieved with the highest doses of thiocyanate that are tolerated by the animals. However, with 100 mg. or more of sodium perchlorate ($NaClO_4$) active uptake of iodide by the rat thyroid can be prevented (4). It was of interest to determine whether such doses of $NaClO_4$ can also completely discharge "trapped" thyroidal iodide.

MATERIALS AND METHODS

Young male rats of the Sprague-Dawley strain (200–250 gm. body weight) were used. They were maintained on Rockland pellets *ad libitum* and tap water. Organic binding of I^{131} was prevented with a single injection of 12 mg. propylthiouracil (PTU) in solution. In Exp. II and III the rats were also pretreated with daily injections of 20 mg. PTU in suspen-

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TABLE 1. EFFECT OF $NaClO_4$ GIVEN BEFORE OR AFTER I^{131} ON THE THYROID-SERUM RADIOACTIVITY (T/S) RATIO

Exp. No.	No. of rats									T/S ratio*
		I. No pretreatment								
		0	45	105				165–195 min.		
I a	6	PTU_1	I^{131}	—				Kill		15.3±2.1†
b	6	PTU_1+$NaClO_4$	I^{131}	—				Kill		0.33±0.018 } p<.01
c	6	PTU_1	I^{131}	$NaClO_4$				Kill		0.43±0.023 }
		0	45	225				285–315 min.		
d	6	PTU_1	I^{131}	—				Kill		12.8 ±1.5
e	6	PTU_1+$NaClO_4$	I^{131}	—				Kill		0.37±0.017 } p<.01
f	6	PTU_1	I^{131}	$NaClO_4$				Kill		0.57±0.055 }
		II. 20 mg. PTU/day for 9 days								
		0	45	105				165–195 min.		
II a	4	PTU_1	I^{131}	—				Kill		93.1 ±14.8
b	5	PTU_1+$NaClO_4$	I^{131}	—				Kill		0.43±0.042 } p<.01
c	5	PTU_1	I^{131}	$NaClO_4$				Kill		0.85±0.032 }
		0	45	165	225			285–315 min.		
d	7	PTU_1	I^{131}	—	—			Kill		39.1 ±6.8
e	12	PTU_1+$NaClO_4$	I^{131}	—	—			Kill		0.58±0.027
f	6	PTU_1	I^{131}	$NaClO_4$	—			Kill		1.39±0.20 } p<.01‡
g	6	PTU_1	I^{131}	—	$NaClO_4$			Kill		1.05±0.084 }
		0	9	15	20	21	22	23	24–24½ hr.	
h	6	PTU_2+I^{131}	PTU_1	PTU_1	PTU_1	—	—	—	Kill	91.6±7.4
i	6	PTU_2+I^{131}	PTU_1	PTU_1	PTU_2	—	—	$NaClO_4$	Kill	2.3±0.27
j	6	PTU_2+I^{131}	PTU_1	PTU_1	PTU_1	—	$NaClO_4$	—	Kill	2.7±0.27
k	6	PTU_2+I^{131}	PTU_4	PTY_1	PTU_1	$NaClO_4$	—	—	Kill	2.2±0.21
		0		25		45			46–46½ hr.	
l	7	PTU_2+I^{131}		PTU_3		$NaClO_4$			Kill	9.9±1.7

* Radioactivity in 100 mg. thyroid/radioactivity in 0.1 ml. serum.
† Mean±standard error.
‡ Compared with experiment No IIe.
PTU_1=12 mg. PTU in solution.
PTU_2=12 mg. PTU in solution+20 mg. PTU in suspension.
PTU_3+12 mg. PTU in solution+40 mg. PTU in suspension.

sion over a period of 9 days. When rats were sacrificed 24 to 46 hours after I^{131} administration, additional amounts of PTU were given as a safeguard against organification of the isotope. Both $NaClO_4$ and potassium thiocyanate (KSCN) were administered subcutaneously in a single dose of 200 mg. Carrier-free I^{131} was given subcutaneously in doses varying from 2 to 50 μc. Treatment schedules are outlined in Tables 1 and 2. Thyroid/serum radioactivity concentration (T/S) ratios were determined by the technique of VanderLaan and Greer (5).

RESULTS

Results are summarized in Tables 1 and 2. In a previous study (4) a T/S ratio of 0.37 was found in rats whose thyroidal iodide "trap" was rendered non-functional with $NaClO_4$. Inhibition of active I^{131} uptake could therefore be considered essentially complete in Exp. Ib and e, IIb and IIIa, in which $NaClO_4$ administration *preceded* that of I^{131}. In Exp. IIe some escape from the action of $NaClO_4$ seems to have occurred. Injection of $NaClO_4$ *after* I^{131} consistently failed to discharge all "trapped" I^{131}. *Ceteris paribus*, the difference between the T/S ratio obtained with $NaClO_4$ given after I^{131} and that observed when iodide "trapping" was prevented with $NaClO_4$ a) was greater when chronic pretreatment with PTU had activated the thyroidal iodide "trapping" mechanism (compare Exp. I a–c with II a–c and I d–f with II d,f and g); b) increased with the length of sojourn of I^{131} in the body (compare Exp. II b–e with II i–l); and c) was not significantly affected by the time elapsed between $NaClO_4$ injection and sacrifice (*vide* Exp. II f,g and i–k).

KSCN was not able to eliminate the thyroid-serum radioiodide gradient. There was no significant difference between the T/S ratio obtained with KSCN injected before or 2 hours after I^{131} (Table 2). However, when KSCN was administered 22 hours after

TABLE 2. EFFECT OF $NaClO_4$ AND KSCN GIVEN BEFORE OR AFTER I^{131} ON THE THYROIDAL IODIDE "TRAP"

Exp. no.	No. of rats	20 mg. PTU/day for 0 days					T/S ratio*	Concentration of "trapped" I^{131} retained in thyroid†
		0		45		105–135 min.		
III a	6	PTU_1+$NaClO_4$		I^{131}		Kill	0.41±0.014‡	—
b	6	PTU_1+KSCN		I^{131}		Kill	1.4 ±0.10	—
		0		45	165	285–315 min.		
c	5	PTU_1		I^{131}	$NaClO_4$	Kill	0.79±0.06	3475±126
d	6	PTU_1		I^{131}	KSCN	Kill	1.6 ±0.10	—
		0	45 min.	5	22	24–24½ hr.		
e	6	PTU_2	I^{131}	PTU_2	$NaClO_4$	Kill	1.8 ±0.30	3470±383
f	6	PTU_2	I^{131}	PTU_2	KSCN	Kill	4.2 ±0.24	—

* Radioactivity in 100 mg. thyroid/radioactivity in 0.1 ml. serum.
† Counts/min/100 mg. thyroid minus counts/min/0.041 ml serum. The rats in Exp. III c and e received simultaneously a dose of I^{131} with an activity of 10.301×10^3 counts/min. The thyroidal counts in Exp. III e were corrected for physical decay.
‡ Mean ± stand. err.

I^{131}, it failed to reduce the T/S ratio as drastically as it did when it was given prior to or sooner after I^{131}.

Exp. III, a, c and e were designed to answer the question whether the concentration of "trapped" thyroidal I^{131} not dischargeable with $NaClO_4$ actually increases with time or whether the observed progressive rise in the T/S ratio is merely due to a decline of serum I^{131} levels while the non-dischargeable I^{131} concentration in the thyroid remains constant or falls less rapidly. In view of the results of Exp. III a, 0.41 × the concentration of serum I^{131} was regarded as the concentration of thyroidal diffused I^{131}. The concentration of non-dischargeable "trapped" I^{131} in the thyroid was computed by subtracting the number of counts due to diffused I^{131} from the total number of counts (per 100 mg. of gland). Table 2 indicates that an actual accumulation of non-dischargeable "trapped" I^{131} in the thyroid did not take place between 4 and 24 hours after I^{131} administration, and that the concomitant rise in the T/S ratio must have been due to the loss of serum I^{131}.

To obtain information regarding the chemical nature of the non-dischargeable "trapped" I^{131}, single thyroid lobes were pooled from the rats of Exp. II i,j and k, respectively, and were homogenized in 0.9% NaCl. The homogenates were shipped in the frozen state to Dr. Jack Gross for chromatographic and electrophoretic analysis.[2] Chromatograms were done in butanol-dioxan-ammonia and paper electrophoresis of aliquots in Veronal buffer at pH 8.6. Scans of the chromatograms revealed 2 peaks of radioactivity, one corresponding with the region of the iodide carrier and the other at the origin. The latter was presumably due to an iodinated protein. The amount of activity in the "protein-bound" fraction increased relative to that of the "iodide" region from Exp. II i to k, i.e., *pari passu* with the duration of $NaClO_4$ action. Electrophoretically the "protein-bound" fraction showed very little mobility and thereby markedly differed from rat thyroglobulin (6).

DISCUSSION

Under the conditions of our experiments most but not all of the I^{131} taken up by the PTU-blocked rat thyroid could be discharged with $NaClO_4$. The concentration of the non-dischargeable fraction appeared to be determined by the thyroidal concentration of

[2] We are greatly indebted to Dr. Jack Gross, Department of Anatomy, State University of New York College of Medicine at New York for these analyses and his generous permission to quote their results.

I^{131} before the administration of $NaClO_4$. The ratio of non-dischargeable/dischargeable I^{131} increased with time, apparently because the dischargeable fraction, which was presumably in equilibrium with serum radioiodide, decreased in concentration as the serum radioiodide level fell, whereas the non-dischargeable fraction did not. No conclusion could be drawn as to the rate of accumulation of thyroidal non-dischargeable I^{131}, except that it did not increase in concentration between 4 and 24 hours after the injection of I^{131}.

Interpretations of the chemical nature of "trapped" I^{131} not dischargeable with $NaClO_4$ must be considered tentative. It evidently is not thyroglobulin-I^{131} formed because of incomplete inhibition of thyroid hormone synthesis by PTU. Taurog *et al.* (7) have found that all thyroidal I^{131} of rats injected with the isotope after chronic PTU treatment behaved as iodide chromatographically. In the chromatograms of extracts prepared from the thyroids of rats used in Exp. II i the "iodide" peak was much more prominent than the "protein-bound I^{131}" peak. However, as $NaClO_4$ was allowed to act for longer periods (Exp. II j and k) the height of the latter peak progressively increased at the expense of the "iodide" peak. It is therefore possible that the non-thyroglobulin "protein-bound" I^{131} found after $NaClO_4$ treatment was actually the result of an (oxidative?) effect of $NaClO_4$ on the thyroidal I^{131} which it did not discharge. Further work will have to decide whether the I^{131} fraction in the thyroids of PTU treated rats which is neither discharged by $NaClO_4$ nor in equilibrium with serum radioiodide is *iodide-I^{131}* contained in a second thyroidal iodide compartment, and if so, whether "trapped" iodide must pass through this compartment in the course of thyroid hormone formation when organic binding of iodine is not blocked. The possibility also has to be considered that the non-dischargeable "trapped" I^{131} fraction arises through an interaction of PTU and I^{131} within the thyroid and therefore has no physiologic significance.

SUMMARY

A dose of $NaClO_4$ which prevents active uptake of I^{131} by the thyroids of propylthiouracil treated rats fails to discharge all "trapped" I^{131} when given after I^{131} administration. The proportion of non-dischargeable/dischargeable I^{131} is greater the longer the interval between the injection of I^{131} and $NaClO_4$. The non-dischargeable I^{131} does not appear to be bound to thyroglobulin.

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SHAW ADRENALINE TEST[1]

While agreeing with the findings of Nuzum and Bischoff (1), who were unable to confirm the statement of Kroneberg and Schümann (2) that the ratio of hydrolyzed to non-hydrolyzed catechol as determined by the Shaw test is increased in the urine of hypertensive individuals, Raab and Gigee (3) challenge the specificity of the Shaw so-called specificity test as applied to adrenaline and noradrenaline.

Originally Shaw (4), confirmed by West (5), stated that noradrenaline did not give the specific adrenaline test (increase in color when alkali is added before the arsenomolybdic acid reduction). Verily (6) and Raab and Gigee (7) did not concur.

It occurred to us that this disagreement might be due to the presence of adrenaline as an impurity in the noradrenaline samples used by those who could not confirm Shaw.

We have therefore reinvestigated the specific and nonspecific Shaw tests. A sample of adrenaline, Lot No. 852X52, was obtained from Parke, Davis and Company. From the information received it contained less than 0.1% noradrenaline. l-noradrenaline bitartrate, Lot No. TuE185a, was obtained from Sterling-Winthrop Company. For the separation of the d and l forms see Tullar (8). A sample of Delta Company noradrenaline hydrochloride was purchased on the open market.

In our hands adrenaline gave a 250% increase in color, while the two samples of noradrenaline gave respectively 20% (Winthrop) and 29% increases in color (11 experiments). It required 50 times as much noradrenaline as adrenaline to produce the same increase in color. Since the two noradrenaline samples gave different increases in color, it is conceivable that the noradrenaline samples could contain in the order of 2% adrenaline as an impurity, which would not be detected by elementary analysis and could account for the color increase. Our results indicate the test is specific for practical purposes, even if pure noradrenaline should subsequently prove to give a slight color increase. We found that Nelson's (9) color reagent may be used in Shaw's test. In low concentrations (.01 gamma adrenaline per test) Nelson's reagent was nine times as sensitive as Shaw's color reagent.

The adrenaline used by Verily was natural adrenaline obtained from Allen and Hanburgs Ltd., London, the noradrenaline was a racemic mixture obtained from Schauman and a sample of l arterenol obtained from Tainter. Verily found no difference in response between the two samples of noradrenaline. In the range of low concentration Verily found that adrenaline had 2.5 times the intensity of noradrenaline in the nonspecific Shaw test. It required 5 times as much noradrenaline as adrenaline to give the same color increase in the specific Shaw tests. Since our results indicated a 50-fold concentration of noradrenaline would be required, our results are obviously in disagreement with Verily.

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ENDOCRINOLOGY—ACHIEVEMENT AND CHALLENGE*

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THROUGH the years endocrinology has contributed in a very significant degree to the solution of many medical problems. These contributions, to cite a few, have served to ease human suffering, to provide health where disabling conditions had existed, to prolong life, and to enable the childless to experience the joy of parenthood. The list of achievements represents one of the most glorious in all of medical history.

Let us take a little closer look at some of these accomplishments. Their recitation, together with the men responsible for them, would have the aspect of being a membership list of our Society and includes notable contributions by those devoted to the laboratory and clinic alike. I shall not dwell at length on these, because, important as they are, they do not represent the principal reason I have for considering them this evening. I wish simply to remind you of some of the things that have been done and in that process set the stage for a challenge that faces us head-on and demands our earnest attention as has no other problem in all history.

You are all aware, for example, of the knowledge that has come to us concerning the chemistry, physiology and diseases of the thyroid gland. These discoveries during the last 40 years have given us invaluable instruction in the handling of overt thyroid disease and in the manner in which disturbed thyroid function exerts profound influence upon many other activities of the human physiologic economy. And we may be very sure that researches now in progress will reveal new facts of outstanding importance, facts which will influence in notable fashion many aspects of human well being.

No less significant have been the studies directed toward the understanding of the chemistry, physiology, and treatment of pancreatic disease. Through the years most of us have come to accept, quite as a matter of fact, the role of insulin in the control of diabetes mellitus as we have the terrific impact it has had upon the extension of life in individuals of all ages. Our knowledge of the pancreas has been the product of studies by many accomplished investigators, and the work goes on at an accelerated pace as we learn of the participation of other endocrine substances of both pancreatic and extrapancreatic origin in the metabolism of carbohydrates.

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* Presidential Address, Endocrine Society, delivered at Chicago, Illinois on June 8, 1956.

Mark these words, such studies have and will in even greater measure continue to have enormous influence upon the life and physiologic economy of mankind.

And what of the adrenal and the fascinating secrets about its manifold functions that have been revealed and placed in the service of man's health? The story would be long and even now is far from complete. It began, as our illustrious past-president so eloquently recalled to us one year ago, a full century past in the observations of Addison and Brown-Sequard. After passing through decades of doldrums and a shift of focus from the adrenal cortex to the medulla, during which time fundamental observations led to the recognition, the isolation, and finally the synthesis of epinephrine, interest in the cortex revived and has undergone progressive acceleration during the last twenty-five years. And well it might, because research on the adrenal cortex has been rewarding both in the revelation of amazing information and in the application of that information to many areas of human suffering. Perhaps in no other area of medicine is the significant role played by hormonal substances in non-endocrine, as well as endocrine, disturbances so well illustrated.

Thus the first studies, which had their roots in the relatively rare disease described by Thomas Addison, have gone far beyond their limited origins and now encompass and find their applications in diseases of virtually every system and organ of the body. Who might have guessed two score years ago, when pioneer studies on the extraction of active principles from the adrenal cortex were coming to fruition, that today we would be using those substances and their derivatives to treat diseases such as those of the joints and bones, the kidneys, the skin, the eye, the vascular system, the reproductive system, cancer and many others? In perhaps no other area of medical research have the combined efforts of the chemist, the physiologist, and the clinician provided solutions to so varied an assortment of problems.

And so goes the role of honor through the less glamorous, but, in its more limited scope, equally significant story of the parathyroid glands and the many-sided saga of the hypophysis, which gland, after limited application, is assuming a position of increasing promise throughout medicine to the gonads and their associated structures. Here the contributions have been less in the nature of solving problems associated with serious diseases, although some grave disorders have felt the impact of research in the field of the sex steroids, and more in the area of improving the fertility of individuals, correcting deficiencies in the reproductive organs, and postponing some processes of ageing. Thus many infertile males and females have been benefited by researches in this field, and many pregnancies, otherwise doomed to early cessation, have been salvaged. Furthermore, I feel certain that as our understanding of the true nature of infertile states progresses and we find such bases of interpretation of these states as locally deranged

metabolic functions and defective enzyme systems, we will be in a position to correct other infertile conditions, and thus help many more of the two million odd infertile couples, in our country alone, to bear children.

I now approach the principal point of this address when I say that in all of these achievements of endocrinology, we have been motivated by the objectives common to all medical science, the prevention and correction of disease, the salvage of life in the unborn, the newborn and the aged, and the improvement of man's health and well-being. In so doing, endocrinology cuts squarely across virtually every area of medicine and provides an enormous influence on the lives of individuals of all ages. The total effect, I am sure you all will agree, is one of very great proportions. We have been instrumental in furthering the creation of new lives where none would otherwise have existed, of salvaging pregnancies that would have terminated, of thwarting diseases which would have been fatal in the newborn, the infant, the child and the adult, and finally of extending significantly the span of life. In most respects this impact of advances in endocrinology upon man's life has been similar to that provided by other areas of medical research, all of which taken together have in a relatively short time had an enormous effect upon the population of the world. Our specialty has contributed much to the present state of rapid growth in numbers, but many other branches of biological and medical research have even greater shares in this result. The antibiotics, antimalarials, pesticides, improved public health measures, etc. have been potent influences in all parts of the world so that death rates have tumbled in areas where endocrinological advances probably have had, as yet, little or no application. This consideration, however, is not truly significant as far as the total picture is concerned.

I should like to have you take a look at that picture—one which all of us have had a hand in bringing into being and one which must be indelibly impressed on our conscious thinking because it represents, my friends, a problem that far outranks any problem facing the world today.

If I shock any of you in these following remarks, I can say only, perhaps you should be shocked, because the truly significant thing that is taking place in the world today is given scant mention in the pronouncements that come to the attention of most of us. It is not the political fate of countries now or destined soon to be created as independent entities, nor the fate of our embattled political parties. Nor is it whether the stock market rises or falls, nor whether this or some other country perfects an intercontinental missile—nor even whether the next World Series is to be a Dodger-Yankee rematch. Of course, all of these are important, particularly the one last mentioned, but they all shrink to a state of absolute insignificance when compared with the truly important matter we must face today. What is this phenomenon? Of course, you must have guessed and guessed correctly —the population of the world is undergoing an explosive growth.

From this point, I tread on ground ill-suited to a work-a-day endocri-

nologist, but I have looked at our own country as well as many other countries sufficiently to have confidence in the calculations of my demographic colleagues. They tell me that the world's population today is in excess of 2.6 billions. Do you realize that only one century ago this mortal sphere contained less than one-half that number? To put it in another way, from the most primitive time of man until the year 1850, the population of the earth was no more than 1.1 billion, but in little more than one hundred years we have applied ourselves to the process of reproduction so effectively that we now number considerably more than twice that total. And that is not all—if we can believe our demographic brethren, maintenance of our present rate of increase will double again the world's inhabitants during the next fifty years. After that it would take no more then thirty to thirty-five years to double once again our population. At this rate the year 2040 would find the world populated by more than eleven billion people. I shall take these calculations no further; the idea of so many people is much too dismal to entertain—even in speculation. Some provisions for control, quite obviously, are imperative and will inevitably be necessary. More than 150 years ago, Malthus wrote of positive checks, which in effect mean simply the death rate, and of preventive checks, that is to say, limitation of the birth rate. As we learn to reduce the death rate, a balance must be sought by reducing the rate of reproduction. The utmost progress in technological developments, despite the optimistic pronouncements we hear from time to time, cannot possibly keep abreast of a population growth which arises from a natural birth rate and a reduction in the rate of death that modern medical developments have provided.

An analysis of the history of human populations shows that population groups undergo what is known as the Demographic Transistion—the transition by gradual stages from cultures with high death rates, high birth rates, and low standards of living to cultures with improved standards of living, low death rates, and birth rates reduced but still far in excess of deaths. Today only 15% of the world's population has completed this transition. The remainder are in various intermediate stages of evolution with the vast majority continuing to remain close to the first stage, but, and herein lies a very grave danger, almost two-thirds of these people have entered or are entering the stage where the death rate has been markedly reduced without a corresponding decrease in births. The consequence of this situation is apparent and reflects the extraordinary effect that modern medical achievements have had upon life in the world today.

The application of these achievements have upset quite completely the predictions for population increases which were made by demographers only a few years ago. I think the following statistics are worth our attention at this point. In 1945 careful calculations were made regarding the anticipated population in 1970 for the world and its various regions. These calculations were based upon available censuses and upon the existing rates of

increase. But look at what happened between 1940 and 1954:

And demographers tell me that they now regard these new estimates (made by the United Nations) as conservative.

It is evident, I am sure, that we are facing an unprecedented increase in our numbers and that without the insertion of checks—positive or preventive—a progressively more serious situation must inevitably arise. Since we are reducing the positive checks by prolonging life, we must pay

	Population 1940	Estimated 1970 (estimated in 1945)	Population 1954	Present Estimate 1970
World	2,200 millions	2,800 millions	2,655 millions	3,153 millions
Asia	1,188 millions	1,567 millions	1,451 millions	1,705 millions
Europe	380 millions	377 millions	407 millions	457 millions
U.S.S.R.	194 millions	242 millions	214 millions	261 millions
Africa	156 millions	218 millions	214 millions	254 millions
North America	144 millions	172 millions	177 millions	209 millions
Latin America	132 millions	203 millions	177 millions	251 millions

heed to the necessity for instituting, on a large scale, more and better preventive checks. In other words, the rate of reproduction must be decreased.

We who inhabit this most fortunate of all countries are extremely prone to regard overpopulation as relating to countries somewhere at the other ends of the earth. We are inclined to think, usually for no longer than it takes to dismiss the thought and turn to something more pleasant, that something should be done about those poor people. It is, of course, true that curbing of nature's positive controls on population has so altered the balance of life and death that the populations of some areas are increasing at the amazing rate of three and four percent each year. But what peoples show such increases? As yet, fortunately, these are not large populations. They are in places like Mauritius, Costa Rica, and Guam. When we think of unusual population increases we are inclined to think of India, China, and Japan. What of them? In the case of China we have no means of making dependable estimates, but we are led to believe that their rate of increase is two percent, that is the difference between a birth rate of 37 per 1000 population and a death rate of 17 per 1000. With a population of at least 600 million, two percent is a sizeable annual increase, 12 millions; but it is not, fortunately, on the order of four or five percent!

What can we say of Japan, that really tight little group of islands with a population of 90 millions? The Japanese are truly realistic people and as such have taken steps to hold their population growth in reasonable check. They are, according to the figures available to us, increasing their numbers by no more than one percent (birth rate—20 per thousand, and death rate —10 per thousand).

And India, that country of teeming millions, what is their plight? At the

present time, they are adding more than 5 millions to their population each year, but this represents an increase of no more than 1.3%, the difference between a birth rate of 40 and a death rate of 27 per 1000.

It is about time to look at our own country, because, my friends, we are inclined to be very smug about matters of this sort. What is happening in our own backyard? We aren't doing so well in spite of our enlightened stage of development. We have reduced our rate of death to about 9 per 1000, a truly commendable achievement. Our birth rate, however, has not been held at a level that would admit us to good standing in the fraternity of those who have arrived at the ultimate stage of the Demographer's Transistion. It stands at approximately 26 per thousand. The difference, birth rate minus death rate, accords us an annual increase of 1.7 percent. At this rate we can expect to have a lot of company in the years to come. Our population at the present time is about 167 millions. Before 25 years have passed, we can anticipate a population of more than 240 millions, and before the turn of the second Christian millennium, if the present rate of increase is maintained, we shall number far in excess of 300 million souls—who will not be, I am disposed to suggest, anywhere near as comfortable as we consider ourselves to be at the present time. And we are concerned about crowded highways and parking problems now!

I think it would be a good idea to consider for a moment the comfort we enjoy today. There are those who would have us believe that the present rate of population growth in this country is in great measure responsible for the enviable economic position we seem to occupy. This shapes up to be a good spot to insert the parable of the Red Queen.

You all will remember Lewis Carroll's delightful tale "Through the Looking Glass" and the Red Queen's remarks about "A slow sort of country. Now, here, you see it takes all the running you can do to keep in the same place. If you want to get somewhere else, you must run twice as fast as that." Well, my friends, that is precisely what we are doing today. Perhaps we should take a long look through our own particular looking glass.

Our gross national product purports to express the value of the national economy's total output of services and goods in terms of current prices. In effect it is a mirror that may be regarded as reflecting our economy as a whole. Just what has happened to it? In 1946 the gross national product was 209.2 billion dollars. It is estimated that in 1955 the figure had increased to 387.4 billion dollars—an increase of 85 percent.

Of course, we should be properly elated about such a performance, and well we might be, if we accept the figures as they are presented and without examining the total picture. When we do peer deeper into the looking glass, however, we find that the population sharing the gross national product in 1955 was about 24 millions greater than in 1946. This reduces to 62 percent the actual increase, in per capita terms.

This is not the end by any means. As each of us knows full well, the

purchasing power of our money has a habit of changing—usually to our disadvantage. When we adjust the purchasing power of our dollars so that this variable is eliminated, the comparison between our gross national product in 1955 and 1946 is reduced to a net gain of 15 percent.

We know, too, that total income is subject to the inroads of taxation. When due consideration is given this factor, and we may finally speak of "personal disposable income," the per capita figure for our gross national product shows an increase of but 5 per cent over 1946—a far cry from the 85 percent that we commenced with and which the heralds of prosperity would have us believe.

I shall not attempt to explain why people as a whole seem to be more than 5 percent better off today than they were ten years ago. This would involve a discussion of such matters as a decreased rate of savings and a vastly increased per capita credit. I am already beyond my depth in figures and I would only pull you in with me. I have said enough, I hope, to suggest to you that a decade of really Herculean effort has provided us with precious little real advancement. We have, indeed, been doing all the running we can do to keep in the same place. And, it may properly be asked, at what cost? The answer to that question is an alarming one.

I am sure we all are aware of the fact that the natural resources of the world are being depleted; but how many of us realize that at the present time we, who number no more than 6 percent of the world's population, are responsible for more than 50 percent of the total annual use of those resources? How long do you suppose we can expect to continue such an improvident rate of exploitation, a rate that must increase if we expect to maintain our existing standards of living for the 3 million people we add to our population each year? An outstanding authority, one month ago, said ten years would be the absolute maximum. Before that time the peoples of the so-called undeveloped countries will have responded to awakening national consciousness and have become aware of what life may actually hold for them. As that occurs, do you suppose that they will permit us to continue our piratical inroads into their resources? Of course not; very properly they will wish to conserve those resources for their own use. Already the wind carries straws that should tell us that our position is precarious and that complacent acceptance of the easy life we have is fatuous nonsense. We cannot hope to continue existing standards of living if we, as a population group, insist on maintaining anything like our present rate of reproduction. Perhaps most of us present at this occasion will not experience the impact of the inevitable change in our way of life, but our children and granchildren will not escape so easily.

I've said almost enough about the dismal prospects we, the people of this country, face. I've said it because we are inclined to think that we need not worry about having too many people. This attitude is the product of many things. We are accustomed to an economy that is favorable; we tend to

be deluded into believing technological advancements can take care of any situation, and we are influenced by the unrealistic attitude of powerful groups who oppose limitation of population growth.

But what of the people of less fortunate countries, the 50 percent and more of the world's population who, we are told, go to bed hungry each night? I am sure there are few intelligent Americans who do not realize that our future is inextricably interwoven with that of people everywhere. Significant developments in New Delhi, Karachi, Djakarta, Moscow, Cairo, and a hundred other places will have increasingly profound effects upon our lives. And there can be no more far-reaching effect than that which has its origin in the pressures engendered by people who are hungry. Basically, this is hunger for food, but other kinds of hunger, too, are making their appearance. The mounting pressures from other peoples for improved economic status must inevitably have a significant effect upon our own economy, so that we may soon find ourselves forced to run *twice* as fast just to stay in the same place.

There are, of course, a number of reasons for the changes that are occurring in the world today, but there is no one reason that is as significant as the pressure of mounting populations. It is logical to ask what may we do to compensate for this flood of human beings as long as we persist in neglecting the obvious method of meeting the issue? If we are gullible we may believe that prosperity increases in proportion to the number of people, and we may have unlimited faith in technology's capacity to solve all problems created by the pressures of mounting populations. I should like to dismiss this fantastic suggestion with no further comment than reference to the remarks I have already made, but suppose we do examine the facts.

The principal problem, of course, is food. There are other considerations such as natural resources, energy requirements, and capital which deserve attention, but first let us look at the problem of food—that is something everyone of us understands.

Thirty percent of the people in the world today have enough to eat. Each year there are 35 million additional people to feed. Those fortunate enough to live in such countries as our own will eat well, for the time being, but will they be able to continue doing so while most of the world goes hungry? So our technologists will show us how to cultivate more land. After all, there are many thousands of untilled acres that might be made productive. In fact, we are told that approximately one billion new acres might be made available by technological subjugation of those parts of the earth that at present are so dry or so cold that no one, except those who realize the serious nature of the problem, even considers them as subjects to cultivation. And mind you, the largest share of this potentially useful land is not located in those parts of the world that need it most. If, however, such land is utilized and if it is possible to increase the productivity of both these newly subjugated areas and of all the presently cultivated land to

the level of that of Europe today, a level of productivity almost twice that obtained in our own country, it would be possible to feed 3.7 billion people on a share-and-share-alike basis. On such a basis the dietary level of underprivileged countries would be raised and that of this country would be reduced to the average of the European countries. This diet would be healthful and adequate, but not very interesting. If, on the same level of production, all the people of Asia would content themselves with what we might call a "calorically adequate" Asiatic diet, while the rest of the nations maintained European standards, the world could support 4.8 billion people. This is a population that is expected in rather less than 50 years. But why go on? And I haven't said a word about the cost of providing such productivity. In case you are interested, the job might be done in 50 years if we are willing to spend 500 billion dollars.

We might, of course, discontinue the improvident American practice of feeding 50 percent of our plant material to animals. The caloric return of this practice, to provide us with steaks and chops, is approximately 10 percent. I shall not carry the point further, because the vast majority of the people on this planet are not as extravagant as we are, but it is predicted that even we shall be obliged within 15 years to reduce by one-third our habit of eating animals as food—and this without regard to any change in the diet of other countries.

If we turn to the ocean as a source of vastly increased amounts of food, we have two possibilities—fish and algae. The best estimates that can be made today offer little encouragement that either of these sources can provide substantial relief to the need for more and more food. And why should we be obliged to resort to a diet of algae?

Strikingly similar analyses might be made for natural resources, energy requirements, and capital. None of these can hope to stand up under the ever increasing pressure of more and more people. Beyond question technological advances now undreamed of will be made and will provide measures that may, temporarily, ease the pressure in one way or another, but they alone cannot avert man's headlong race toward suicide.

We are told that space travel may one day come to reality and enable us to propel our surplus people off to colonize other planets. Perhaps interplanetary travel will become practical and perhaps other planets which provide the circumstances essential for support of human life can be found. If all of these vague possibilities were true today, we would be obliged to despatch into outer space 100,000 people each and every day simply to maintain the population of the world at its present level.

The doubt I have just expressed in the capacity of technology to solve man's problems, no matter what his numbers may be, scarcely agrees with the calculations revealed in a recent feature article in the *New York Times* (May 21, 1956). This article based upon a publication, "Resources of the World," issued from the California Institute of Technology would have us

believe that man's technical skill can provide for any emergency and that the only shortage we need ever fear is a dearth of brain power. It is implied that in the face of as many as 30 billion people there would be no shortage of minerals, meals, or metals.

Articles such as these actually do a great disservice, because they tell part of the truth only. In the case in point, no mention is made of the cost of achieving support for 5, 10, or 20 times the present population of the world. Nor is a picture given of the kind of life the inhabitants of such a world would have. It would be a totally different life than the one we enjoy today, shorn of all luxuries of food, creature comfort, mechanical devices, and perhaps most of all, room to move around. The commodities we accept as commonplace today, and new ones we anticipate in the future, could not be afforded in such a world because the necessities of life would require all our resources and effort and the open spaces we cherish would no longer be open because someone would be occupying them.

The publication, "Resources of the World," upon which the *Times* press release was based, actually was drawn heavily upon in preparing this address. It does not ignore the problems man would face in a world in which the ever-mounting pressures of more and more people would demand all his effort and ingenuity simply to maintain the necessities of life—rather it recognizes those problems and attempts to speculate as to what the situation is likely to be if population is not restricted more effectively than is the case today.

But let us be practical. Why should it be necessary to concern ourselves with fantastic schemes to accommodate vast numbers of human beings when, by the exercise of common sense and ingenuity, we can avert disaster? With the achievements technology has already secured and will continue to secure in the near future, we are in a position to improve the lot of people everywhere and to bring them closer to the higher standards of living we ourselves enjoy, but this will never be possible if populations here and elsewhere continue their present rate of increase. With the exercise of wisdom we might even look forward to an appreciable improvement, in the eugenic sense, of our race. I think we must decide *now* whether we shall bequeath to future generations not only the extraordinary technical advances we have secured in all fields, but *also* a world of so many people that only few can really enjoy those advances. I say we have no right to make the latter bequest! Our purpose should be to provide as much improvement as we can to a relatively small number of people, and after technological advances are applied as widely as possible, to permit future generations to decide whether more people are desirable. In short, we have the choice of running many times as fast even to be close to the same place or running, as the Red Queen suggested, twice as fast and reaching new places.

And so we have arrived at the point of this harrangue, the challenge I should like to present this evening. I have tried to show you that the world

of today faces a problem of unprecedented proportions—it is approaching very rapidly the state where our hopes for a decent life for all mankind will be rendered increasingly more difficult, if not impossible, to achieve. Of course, we very well may see the institution, in the Malthusian sense, of very positive *positive* controls—such as the hydrogen bomb or something even more unequivocally positive. That might take care of the situation, but my demographic colleagues tell me that no more than 5 percent of the world's population lives in the 60-odd communities of more than one million inhabitants. These cities are the most likely targets for weapons of mass destruction, yet if all of them should be wiped out completely, the people remaining elsewhere could replace those so destroyed in less than five years if the present rate of propagation is maintained. Of course, there would be serious effects upon people everywhere if the major cities of the world were so destroyed. Many sorts of positive checks on population growth might ensue, but it should be apparent that the destruction of people by the hundreds of millions would not in itself be a major deterrent to the inexorable increase in our species. The use of the frightful weapons now available to man may, indeed, participate in the ultimate solution of the truly important problem we face today, and there is little reason to doubt that they will eventually be used if the human species does not exert strenuous efforts to curb its reproductive capacities. We may not in this generation experience that catastrophe, but within all too short a time, the pressures of excessive population are almost certain to be such that the existing restraints will be overpowered. Someone will gamble all in the struggle for the remaining resources of the world.

Why should we stand in idleness when something can be done? After all, a major factor in the creation of our paramount problem has been the diligence with which the biological sciences have sought to preserve human life. Now that we have removed, in very large measure, nature's *positive* controls on population growth, we are obliged to seek *preventive* measures.

Modern medical technology in many fields is responsible for reduction in the rates of death and disabling disease and for the provision of improved conditions of health, each of which increases life expectancy at all ages. The release from curbing influences can have no other effect than the acceleration of population growth. As we have noted, it has been the history of population groups that as they reach the final stage in the Demographic Transition birth rates do tend to fall, but we must face the fact that modern medical advances have been applied so promptly and so extensively that the rates of death rather than dropping gradually have fallen very rapidly and in some critical areas are destined to show further significant decreases. In the past, the decline in death rates has been gradual enough to permit the drop in birth rates to keep fairly well abreast, but such is not true today.

We cannot depend upon spontaneous acceptance and application of the conventional methods of controlling fertility as being nearly rapid enough

to prevent an even more explosive population growth than has been projected. Vigorous effort must be put forth to educate people everywhere so that they will understand the perilous nature of this problem, and linked with such effort, there must be provision for ready access to available contraceptive measures. These measures will be acceptable in one form or another to a large number of the people of many countries and can provide formidable assistance in stemming the tide that threatens increasingly to overwhelm us. But it seems true that existing methods of contraception are either not likely to be acceptable or are not practicable as applied to large segments of the populations in some areas. There is a great need for methods involving the physiological control of fertility, methods that are simple, safe, effective, and economical.

I have said before that the biological sciences are responsible in large measure for the present rate of population growth, and we, as endocrinologists, share that responsibility. It is essential that we recognize this fact and do our utmost to provide information that will lead to the formulation of methods which will aid in establishing a balance between the rates of birth and death.

This is the responsibility of all of us who are associated with the biological sciences. I have admitted that our own science, endocrinology, has probably done less to complicate the problem than have some other specialties, but, my friends and colleagues, our group is one of the few which is in the position of being able to do something really positive about the situation. It is within our province, and I think we should count ourselves very fortunate, to learn a great deal more about the endocrine mechanisms of reproduction, to be on the alert constantly for observations which may be of value in unravelling the secrets of the reproductive processes, and wherever possible, to design and focus our efforts to the purpose of learning, by physiologic methods, how to curb man's fertility. Endocrinologists, past and present, have faced up to and answered many difficult challenges. This is, by all odds, the greatest challenge we shall ever need to face.

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Although direct references have not been given in the body of this paper, it may be of interest to some readers to examine in more detail a few of the publications that have been used as source material. Reference to these will provide many other valuable papers and books on the subject.

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